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Human variability in polymorphic CYP2D6 metabolism: Implications for the risk assessment of chemicals in food and emerging designer drugs

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ABSTRACT

The major human cytochrome P450 CYP2D6 isoform enzyme plays important roles in the liver and in the brain with regards to xenobiotic metabolism. Xenobiotics as CYP2D6 substrates include a whole range of pharmaceuticals, pesticides and plant alkaloids to cite but a few. In addition, a number of endogenous compounds have been shown to be substrates of CYP2D6 including trace amines in the brain such as tyramine and 5-methoxytryptamine as well as anandamide and progesterone. Because of the polymorphic nature of CYP2D6, considerable inter-phenotypic and inter-ethnic differences in the pharmacokinetics (PK/TK) and metabolism of CYP2D6 substrates exist with potential consequences on the pharmacology and toxicity of chemicals. Here, large extensive literature searches have been performed to collect PK data from published human studies for a wide range of pharmaceutical probe substrates and investigate human variability in CYP2D6 metabolism. The computed kinetic parameters resulted in the largest open source database, quantifying inter-phenotypic differences for the kinetics of CYP2D6 probe substrates in Caucasian and Asian populations, to date. The database is available in supplementary material (CYP2D6 DB) and EFSA knowledge junction (DOI to added). Subsequently, meta-analyses using a hierarchical Bayesian model for markers of chronic oral exposure (oral clearance, area under the plasma concentration time curve) and acute oral exposure (maximum plasma concentration (C_{max}) provided estimates of inter-phenotypic differences and CYP2D6-related uncertainty factors (UFs) for chemical risk assessment in Caucasian and Asian populations classified as ultra-rapid (UM), extensive (EMs), intermediate (IMs) and poor metabolisers (PMs). The model allowed the integration of inter-individual (i.e. inter-phenotypic and inter-ethnic), inter-compound and inter-study variability together with uncertainty in each PK parameter. Key findings include 1. Higher frequencies of PMs in Caucasian populations compared to Asian populations (>8% vs 1–2%) for which EM and IM were the most frequent phenotype. 2. Large inter-phenotypic differences in PK parameters for Caucasian EMs (coefficients of variation (CV) > 50%) compared with Caucasian PMs and Asian EMs and IMs (i.e. CV < 40%). 3. Inter-phenotypic PK differences between EMs and PMs in Caucasian populations increase with the quantitative contribution of CYP2D6 for the metabolism (*f_m*) for a range of substrates (*f_m*_{CYP2D6} range: 20–95% of dose) (range: 1–54) to a much larger extent than those for Asian populations (range: 1–4). 4. Exponential meta-regressions between *f_m*_{CYP2D6} in EMs and inter-phenotypic differences were also shown to differ between Caucasian and Asian populations as well as CYP2D6-related UFs. Finally, implications of these results for the risk assessment of food chemicals and emerging designer drugs of public health concern, as CYP2D6 substrates, are highlighted and include the integration of *in vitro* metabolism data and CYP2D6-variability distributions for the development of quantitative *in vitro in vivo* extrapolation models.

1. Introduction

The quantification of inter-individual differences in the

pharmacokinetics (PK)/ toxicokinetics (TK) and the pharmacodynamics (PD/TD) of xenobiotics provides a scientific basis to bridge external dose, internal dose (absorption, distribution, metabolism and

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excretion (ADME)) and its toxicological consequences for a given chemical. In addition, it allows to refine methods applied in chemical risk assessment through integrating mechanistic understanding and implementation of New Approach methodologies (NAMs). Over the last three decades, such quantitative means have supported international scientific advisory bodies and risk assessors to refine uncertainty factors (UFs) applied for the derivation of safe levels of exposure in humans for xenobiotics including pharmaceuticals, food and feed additives, pesticides, food contact materials, novel foods and contaminants of natural or anthropogenic origin (e.g. mycotoxins, marine biotoxins, persistent organic pollutants etc.). Historically, a 100-fold default UF has been applied to apical endpoints as reference points (RP) or points of departure (POD) from sub-chronic studies in test species (rat, mice, rabbit, dog) i.e. no-observed adverse effect levels (NOAEL), benchmark dose upper confidence limits (BMDL) to derive health-based guidance values for non-cancer effects including acceptable or tolerable daily intakes (ADI, TDI) (Truhaut, 1991). This 100-fold default UF has been rationalised to account for interspecies differences (10-fold) and human variability (10-fold) further subdivided into two equal default uncertainty factors ($10^{0.5} = 3.16$) to allow inter-individual differences in TK and TD dimensions. These sub-divisions have been essential to integrate chemical-specific TK and TD data into physiologically-based TK and TD models and refine default UFs as pathway-related UFs when metabolic pathways and elimination patterns are available (phase I, phase II, transporters, renal excretion) or ideally chemical-specific adjustment factors (CSAF) (Bhat et al., 2017; Calabrese, 1985; Dorne, 2010; Dorne et al., 2001a; Dorne et al., 2001b; Dorne et al., 2003; Dorne et al., 2004; Dorne et al., 2005; Dorne et al., 2002; Ginsberg et al., 2002; Renwick and Lazarus, 1998).

Xenobiotic metabolism involves diverse enzymes including the cytochrome P-450 s (CYP) as major ubiquitous phase I enzymes which play a role in the pharmacology and toxicology of a huge plethora of therapeutic drugs, contaminants, pesticides and food additives to cite but a few. Such CYP isoforms are often polymorphic by nature and the characterisation of human evolutionary aspects of their expression and activity profiles has been shown to have a large impact on inter-individual differences in TK and TD processes (Dorne, 2010; Johansson and Ingelman-Sundberg, 2011).

In this context, the highly polymorphic human CYP2D6 gene is a member of the CYP2D gene subfamily and is localised on chromosome 22q13 along with two highly homologous neighbouring pseudogenes (CYP2D7P and CYP2D8P). Over 133 CYP2D6 variant star (*) alleles have been characterised (excluding sub-variants) amongst world populations of different ancestral origins and such information is available from the PharmVar database and the Human CYP450 allele nomenclature database (<https://www.pharmvar.org/>) (Taylor et al., 2020). Historically, four different CYP2D6 phenotypes have been recognised: ultra-rapid metabolisers (UM), extensive metabolisers (EM), intermediate metabolisers (IM) and poor metabolisers (PM), even though the polyallelic nature of CYP2D6 has been recognised particularly in Caucasian populations (i.e. from 0 to 13 copies of the gene) and associated with prevalence of PM (approx. 8%). In contrast, Asian populations are known to have low prevalence of PM (1%) and high prevalence of IM (between 35 and 55%) compared with Caucasian populations (less than 2%) (Yang et al., 2017; Zhou, 2009). Current categorisation of CYP2D6 metaboliser status is based on activity scores of known haplotypes as a useful tool to classify the function of individual haplotypes into an overall predicted metaboliser status for a given diplotype in an individual. Activity scores for the different phenotypes include 1.25–2.25 for EMs, > 2.25 for UMs, 0.25–1 for IMs 0 for PMs. Such phenotypes are associated with variable allele frequencies across world populations ranging from 67 to 90% in EMs 1–21% for UMs, 0.4–11% for IMs and 0.4–5.4% for PMs (Gaedigk et al., 2017; Taylor et al., 2020). The variant coding for the CYP2D6*4 allele has been found as the most frequent one, leading to the PM phenotype in Caucasian populations whereas CYP2D6*10 is responsible for a decreased function

in Asian populations. This allele rarely occurs in African populations and it has been hypothesised to be the likely outcome of the introgression of a Neanderthal genetic variant in Caucasians since Neanderthals have been shown to be PMs (Ingelman-Sundberg et al., 2014; Steffens et al., 2019; Viviani et al., 2020). Relationships between CYP2D6 genotypes and phenotypes across world populations have been recently investigated for the Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines with the most comprehensive available dataset so far (>60,000 subjects, 173 reports) which provides a quantitative tool for the prediction of CYP2D6 phenotype from genotype based on activity score (Gaedigk et al., 2017).

CYP2D6 is expressed in the human liver (2–4% of total hepatic CYP) as well as in the brain, where it plays important physiological functions with regards to endogenous and xenobiotic metabolism (Taylor et al., 2020). In the brain, CYP2D6 expression is widely expressed in numerous neurons in the brain regions including those within the thalamus, hypothalamus, pyramidal cells of the cortex and hippocampus, substantia nigra, amygdala and Purkinje cells of the cerebellum. CYP2D6 expression varies greatly throughout the life, being very low at birth and gradually increasing with age, until it reaches its highest peak in adults over age 65. Such differences may account for the reduced response to several CYP2D6 CNS acting drugs e.g. desipramine response in older adults and increased sensitivity in infants to codeine (Mann et al., 2012; Voronov et al., 2007). CYP2D6 mRNA and proteins have been shown to be significantly increased in brain in the presence of certain chemicals crossing the blood brain barrier, such as nicotine and ethanol in monkeys, although this has not been shown in the liver. These findings further demonstrate that CYP2D6-derived metabolites of xenobiotics are uniquely present, or formed in greater amounts, in the neocortex and substantia nigra of the human brain at levels exceeding those observed in liver (Bromek et al., 2010; Miksys and Tyndale, 2006; Miller et al., 2014). In addition, variable CYP2D6 activities in the brain has been associated with susceptibility to numerous neurological disorders including neuroleptic malignant syndrome (Kato et al., 2005; Zivković et al., 2010) and to be absent in the brain of Parkinson's and Alzheimer's disease patients (He et al., 2015; Singh et al., 2010; Woo et al., 1999). Recent evidence concludes that CYP2D6 may play a role in the endogenous metabolism for a number of substances and neurotransmitters in the brain including 5-methoxytryptamine (melatonin and serotonin precursor) (Yu et al., 2003), p- and m-tyramine (dopamine precursor) (Bromek et al., 2010), allopregnanolone as a GABA-modulator (Niwa et al., 2008), octopamine (norepinephrine precursor) (Yu et al., 2003; Haduch and Daniel, 2018), hydroxylation of progesterone to four metabolites (2b-OH, 6b-OH, 16a-OH and 21-OH) involved in the formation of other steroids (Hiroi et al., 2001) as well as a number of compounds up- or downregulated in CYP2D6-transgenic mice (Tay-Sontheimer et al., 2014). CYP2D6 also affects the endocannabinoid system within the CNS due to its ability to metabolise anandamide and its derivatives; however, the physiological significance of such metabolic involvement is still unclear (Haduch and Daniel, 2018; Ingelman-Sundberg et al., 2014). Stingl and Viviani (2015) in a meta-analysis focused on antipsychotics and antidepressants and genotype-based dosing recommendations for CYP2D6/CYP2C19, highlighted that beside the CYP2D6 role in hepatic drug metabolism, CYP2D6 polymorphic expression in the brain and role in biotransformation of endogenous substrates may therefore modulate brain metabolism and affect the function of the neural substrates of cognition and emotion. Hence, CYP2D6 phenotyping using endogenous substrates is a potential alternative to the use of CYP2D6 probe substrates. This has been explored using a global metabolomic approach in humans through the detection of an endogenous urinary biomarker (m/z 444.3102) associated with CYP2D6 activity in 189 pediatric patients (Tay-Sontheimer et al., 2014). Overall, four endogenous biomarkers have been partially studied to date in humans namely Codeine/Morphine identified endogenously in humans, 5-Methoxytryptamine/serotonin, pinoline/6-Hydroxy-1,2,3,4-Tetrahydro-Beta-Carboline and m/z 444.3102. However, available data in

humans are still insufficient to allow CYP2D6 phenotyping with endobiotics and fully characterise the role of CYP2D6 in the metabolism of endogenous substrates (Tay-Sontheimer et al., 2014; Tracy et al., 2016; Magliocco et al., 2019).

CYP2D6 is a major isoform involved in xenobiotic metabolism and over 20–25% of all prescribed drugs and other xenobiotics have been identified as CYP2D6 substrates including antiarrhythmic agents, neuroleptics, antidepressants (tricyclic, tetracyclic and serotonin reuptake inhibitors), b-blockers, opiates as well as pesticides, plant alkaloids and derivatives (e.g. sparteine in lupin, lysergic acid diethylamide (LSD), amphetamine-like designer drugs such MDMA and neurotoxins such as tetrahydroiso-quinolines and b-carbolines 1-methyl-4-phenyl-1,2,3,6-tetrahydro-pyridine (MPTP) (Haduch and Daniel, 2018). A number of authors have hypothesised that the alteration of CYP2D-mediated metabolism induced by endogenous steroids may cause changes in sensitivity to environmental and industrial toxins and carcinogens as well as physiological and pathophysiological processes controlled by biologically active substances (Haduch and Daniel, 2018; Ingelman-Sundberg et al., 2014).

From this body of evidence, investigating human variability in the metabolism and PK of CYP2D6 probe substrates can provide quantitative insights to integrate inter-phenotypic differences in the risk assessment process (Yang et al., 2017; Zhou, 2009). In the early 2000s, relevant meta-analyses have been performed for markers of acute (C_{max}) and chronic exposure (AUC and clearances) and demonstrated large inter-individual kinetic differences across CYP2D6 substrates as well as exponential relationships ($R^2 > 0.80$) between phenotypes (EMs, PMs) and the extent of CYP2D6 metabolism for major and minor substrates of CYP2D6 isoform (10–100% metabolism in EMs) (Chiba et al., 2012; Dorne, 2010; Dorne et al., 2002). However, the database, available at the time, was mostly limited to Caucasian populations and since then a large amount of PK data covering a wide range of CYP2D6 substrates and phenotypes in Caucasian and Asian populations have been published.

A number of recent meta-analyses have been performed to investigate human variability of CYP2D6 substrates. Most studies focused on antipsychotics and antidepressants providing CYP2D6/CYP2C19 genotype-based dosing recommendations and prevent drug toxicity (Stingl and Viviani 2015; Milosavljevic et al. 2021). Other meta-analyses have focused on individual drugs including aripiprazole (Zhang et al. 2019); hydrocodone (Linares et al. 2015); metoprolol (Blake et al. 2013; Meloche et al. 2020; Li et al. 2017); risperidone (Cui et al. 2020; Maruf et al. 2020 Zhang et al. 2020) tamoxifen (Province et al. 2014) (Cronin-Fenton, Damkier, and Lash 2014); tramadol (Wen et al. 2020) and veliparib (Stodtmann et al. 2021); venlafaxine (Lin et al. 2019; Ahmed et al. 2019); vortioxetine (Areberg et al. 2014).

This manuscript investigates inter-phenotypic and inter-ethnic differences in the PK of CYP2D6 probe substrates by means of extensive literature searches and Bayesian hierarchical meta-analysis (Darney, 2019; Wiecek et al., 2019). In addition, modelling of the relationships between quantitative metabolism of CYP2D6 in EMs and inter-phenotypic differences between Caucasian and Asian populations are investigated and CYP2D6-related UFs are derived. Finally, implications of these findings for the risk assessment of food chemicals and emerging designer drugs of public health concern, as CYP2D6 substrates, are highlighted particularly for the integration of human *in vitro* metabolism data and CYP2D6-variability distributions and for the development of quantitative *in vitro in vivo* (Q) (IVIVE) extrapolation models.

2. Material and methods

2.1. Extensive literature searches

Probe substrates for CYP2D6 and oral human PK studies reporting markers of acute (maximum plasma concentration (C_{max}) and chronic exposure (area-under the plasma concentration curve (AUC) or

clearance)) in phenotyped individuals (EMs, IMs, PMs) from a range of world populations from different geographical ancestry were identified through two large extensive literature searches (ELS) and were performed by two independent reviewers (January 1989 to July 31st 2020) using online databases (PubMed and Scopus). These ELS were supplemented with available PK historical databases further supporting the identification of CYP2D6 probe substrates using 1. *In vitro* metabolism studies identifying CYP2D6- in EMs 2. Urinary excretion profiles reflecting *in vivo* metabolism expressed as the fraction metabolised by CYP2D6 in EMs (Southampton University and NOMIRACLE databases) (Dorne et al., 2002; Løkke, 2010).

Primary screening of the literature was carried out on titles and abstracts, after removal of duplicates and the following exclusion criteria were applied to studies reporting: 1. PK data for non-phenotyped human individuals or animal species, 2. *In vitro* data, 3. Population modelling including Physiologically-based modelling, 4. PK studies on unhealthy individuals, 5. PK studies on substrates metabolised by other CYP isoforms than CYP2D6, 6. Human PK studies using analytical methods with low sensitivity and specificity (radioimmunoassay). 7. Studies dealing with pharmacodynamic data only or therapeutic drug development.

2.2. Data collection for CYP2D6-phenotyped populations

Data collection was performed for available human PK studies for CYP2D6 probe substrates reporting markers of acute and chronic exposure (C_{max} , AUC, clearance) in EMs, IMs, PMs and UMs from populations of different geographical ancestry. The selected human PK studies were imported into an EndNote database and a second screening was performed on each full text article to evaluate the methodological quality including design, analysis and reporting of the study. Each PK study reporting markers of oral acute and chronic exposure were then computed into a MS-Excel database for each phenotype and subgroups of the population.

Data from individual PK studies reporting phenotypes were also harmonised to perform meta-analyses for each phenotype i.e. UMs, EMs, IMs and PMs. In some cases, genotypes were reported (i.e. combination of alleles) and were assigned to phenotype groups using the most common associations between phenotype groups and alleles from range of studies reflecting inter-phenotypic and differences between populations of different geographical ancestry (Cui et al., 2007; Curry et al., 2007; Gan et al., 2007; Garcia-Quetglas et al., 2007; Gassó et al., 2013; Kirchheiner et al., 2008; Kuhn et al., 2007; Malhotra et al., 2008; Malhotra et al., 2009; Nichols et al., 2011; Pedersen et al., 2006; Samer et al., 2010; Sharma et al., 2010; Slanař et al., 2007; Yoo et al., 2012) according to the following:

- The PM phenotype was associated with homozygosity for CYP2D6 alleles with no activity (i.e.*3, *4,*5,*6,*7,*8,*11,*14,*15,*18,*19,*20,*21,*40).
- The IM was associated with homozygosity with decreased CYP2D6 activity alleles (i.e.*9,*10,*17,*21,*36,*29,*41,*45,*46) or heterozygosity with one decreased activity alleles and one non activity alleles.
- The EM phenotype was associated with homozygosity with normal CYP2D6 activity alleles (i.e.*1,*2) or heterozygosity with one decreased activity alleles and one non activity alleles.
- The UM phenotype was associated with individuals with at least one active CYP2D6 gene duplication.

2.3. Data standardisation

Data standardisation was required for each individual PK parameter and unit while correcting to dose and body weight (b.w) i.e. Area under the plasma-concentration curve (AUC) (ng.h/ml/mg.kg), plasma peak concentration (C_{max}) ng/ml/ mg.kg) and clearance (ml/h/kg). Body

weight correction from the parameters were performed using mean body weight (kg) recorded from the study when available or allocating them to the country of origin (Walpole et al., 2012). Such data standardisation allowed to perform the meta-analyses using harmonised metrics.

Data from individual PK studies were mostly reported as either arithmetic mean (X) and standard deviations (SD) for a normal distribution or geometric mean (GM) and geometric standard deviation (GSD) for a lognormal distribution. Since PK data are generally recognised to be lognormally distributed, all PK data were harmonised to GM and GSD. When such measures were not reported, these were estimated for each individual study using the following equations (Dorne et al., 2001b; Naumann et al., 1997; Renwick and Lazarus, 1998):

$$GM = \frac{X}{\sqrt{1 + CV_N^2}}$$

$$GSD = \exp\left(\sqrt{\ln(1 + CV_N^2)}\right)$$

Where CV_N is the coefficient of variation for normally distributed data given by:

$$CV_N = \frac{SD}{X}$$

2.4. Hierarchical Bayesian meta-analysis of inter-phenotypic differences in CYP2D6 metabolism

A hierarchical Bayesian model, developed in the R freeware, was implemented for each PK parameter (i.e. CL, AUC, Cmax) and each phenotype (i.e. PMs and EMs for Caucasian populations, IMs and EMs for Asian populations) as previously described (Wiecek et al., 2019; Darney et al., 2019). When available, prior data for CYP2D6 variability were used (Dorne et al., 2002), otherwise non-informative priors were applied. Outcome of the meta-analysis models provided variability and uncertainty distributions reflecting inter-phenotypic differences in CYP2D6 metabolism for acute and chronic PK parameters as median values, 95% confidence intervals and coefficients of variation (CVs) estimated as follows:

$$CV = \sqrt{\exp\left(\ln\left(\sqrt{\exp(1/\tau_j)}\right)\right)^2 - 1}$$

where τ_j is the inter-individual differences of the activity for a substrate 'j'.

2.5. Fraction of dose metabolised by CYP2D6 in extensive metabolisers and derivation of CYP2D6-related Uncertainty factors

2.5.1. Fraction of dose metabolised by CYP2D6 in EMs

For each probe substrate, the fraction of the dose metabolised by CYP2D6 in EMs (fm_{CYP2D6}) was calculated using the relationship proposed by Ito et al. (2005):

$$fm_{CYP2D6} = 1 - \frac{CL_{PM}}{CL_{EM}} = 1 - \frac{AUC_{EM}}{AUC_{PM}} \quad (1)$$

2.5.2. CYP2D6-related uncertainty factors for inter-phenotypic differences in pharmacokinetics

CYP2D6-related UFs for each phenotype (UM, EM, IM, PM), in populations of different geographical ancestry, were derived assuming a unimodal distribution as the ratio between the median of the distribution and a given percentile (i.e. 95th or 97.5th). For inter-phenotypic differences, CYP2D6-related UFs were calculated as the ratio between the median of the reference group (i.e. EM) and the given percentile of the UM, PM and IM phenotypes (Dorne et al., 2002; Wiecek et al., 2019). As previously described, medians and 95th confidence intervals around the UFs were derived through simulating 10 000 UF values using

Markov Chain Monte Carlo (MCMC) simulations.

2.5.3. Meta-regression between the fraction of dose metabolised by CYP2D6 in EMs and inter-phenotypic differences in pharmacokinetics

Relationships between the fraction of dose metabolised by CYP2D6 for each probe substrate in EMs and inter-phenotypic differences in PK parameters (AUC, CL, Cmax) were modelled through meta-regression for each population and CYP2D6 phenotype.

2.6. Software

References from the ELS were computed in EndNote (X8) files. All statistical analyses and graphical display of the data were performed using R (version 3.5) while the Bayesian modelling was implemented within Jags (4.2.0) (Plummer, 2003). All R codes applied for the meta-analyses are available open source (Darney et al., 2019; Wiecek et al., 2019). Meta-regressions between the fraction of dose metabolised by CYP2D6 in EMs and inter-phenotypic differences for each PK parameter (AUC, CL, Cmax) were generated in the Benchmark dose modelling tool of the European Food Safety Authority (EFSA) (<https://shiny-efsa.openanalytics.eu/app/bmd>).

3. Results and discussion

3.1. Extensive literature searches and data collection

Results from the extensive literature searches are illustrated in a prisma diagram (Fig. 1) for which a total of 3654 records were identified in the first screening and a total of 130 papers were included in the database. All individual datasets are presented in the supplementary material as an excel spreadsheet and are published on EFSA Knowledge Junction under DOI XXX.

Overall, results highlight that most PK studies were available for phenotyped adult Caucasian and Asian populations and meta-analyses were performed for these populations. Very few studies were available for the PK of CYP2D6 substrates in Caucasian IMs (7 studies, 6 substrates) and for PM Asian individuals (3 studies, 3 substrates) which is consistent with the very low prevalence of both phenotype in these populations (1%). In addition, PK data for phenotyped individuals in other human sub-populations from Caucasian or Asian origin (i.e. elderly, children, neonates) were very scarce and did not allow to perform a meta-analysis. Consequently, meta-analysis for Caucasian IMs and Asian PMs were not performed due to insufficient number of individuals. For all remaining subpopulations, Bayesian meta-analyses were performed using data from 80 studies (AUC) and 29 CYP2D6 substrates in Caucasian healthy adult EMs and 46 studies for 21 CYP2D6 substrates in Caucasian PMs respectively. For Asian populations, the meta-analyses were performed using data for 58 studies and 21 substrates in EMs and 31 studies and 18 substrates in IMs respectively.

3.2. Inter-phenotypic differences in CYP2D6 metabolism and CYP2D6-related uncertainty factors

3.2.1. Inter-phenotypic differences for CYP2D6 metabolism in Caucasian populations

Variability in PK parameters reflecting CYP2D6 metabolism for markers of chronic exposure and CYP2D6-related UFs in Caucasian EMs are illustrated in table 1 and table 2 for AUC and CL. Number of studies (Ns), samples size (n), meta-analysed geometric means (GM), coefficients of variation (CV) and CYP2D6-related UFs 95 and 97.5 and respective 95th confidence intervals [CI] are reported.

Overall, variability in AUC and clearance parameters for Caucasian EMs ranged from 4% to 109% (AUC) and 5% to 102%. For major CYP2D6 substrates ($fm_{CYP2D6} > 75\%$), variability in EMs was large for both AUC and clearance (mean $> 50\%$; range: 50–100%). CYP2D6-related UFs ranged for AUCs [1.1–4.3 (95th centile) and 1.1–5.8

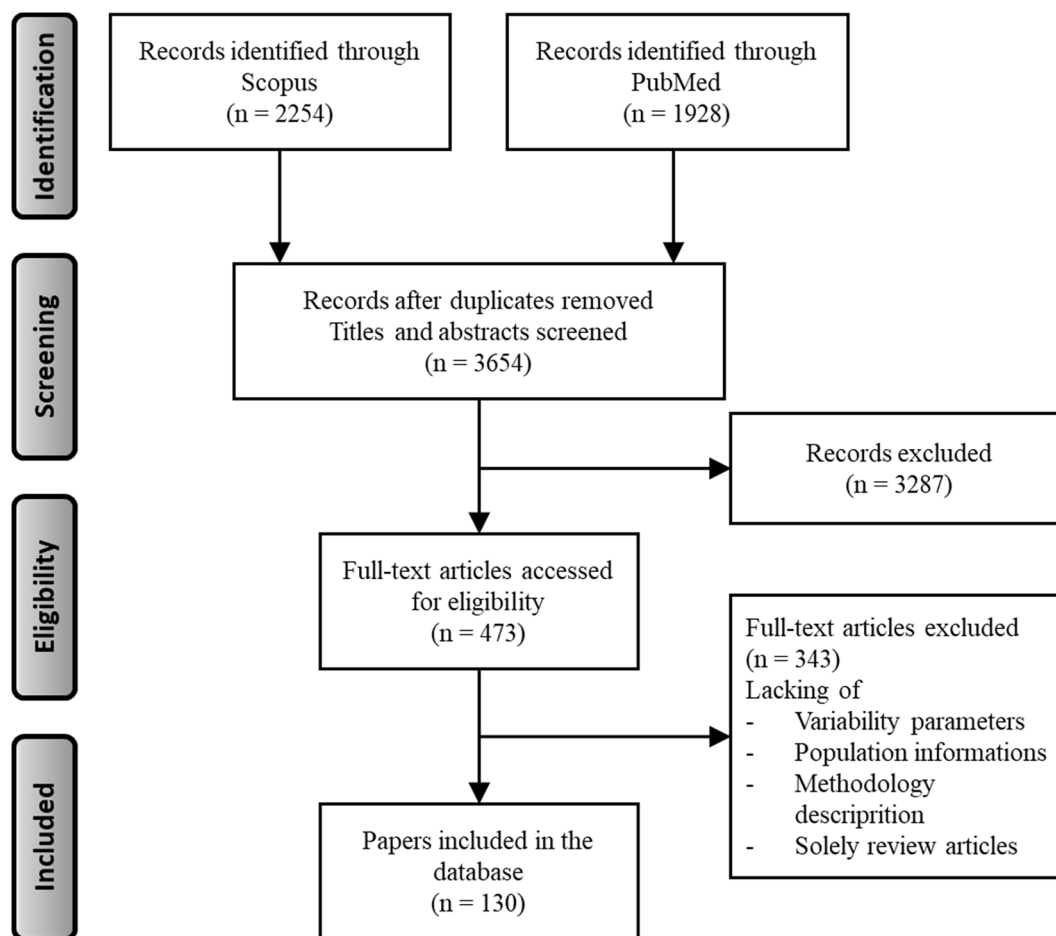


Fig. 1. Prisma diagram illustrating the results of the extensive literature search on pharmacokinetic studies for human CYP2D6 probe substrates.

(97.5th centile)] and for clearances [1.1–9.5 (95th centile) and 1.1–14.2 (97.5th centile)]. Consistently, the CYP2D6-related UFs were mostly below the default TK UF for minor CYP2D6 probe substrates ($fm_{CYP2D6} < 75\%$) whereas for major probe substrates ($fm_{CYP2D6} > 75\%$), CYP2D6-related UFs were most often within or above the TK default UF to cover 95% and 97.5% of EMs.

Variability in PK parameters reflecting CYP2D6 metabolism for parameters of chronic exposure in Caucasian PMs and inter-phenotypic differences between EMs and PMs are reported in table 3 and table 4. Overall, variability in PK parameters for PMs ranged from 8 to 80% across AUCs, Clearances and substrates. Overall, differences in internal dose between PMs and EMs were variable with differences ranging from 1 to 7-fold for substrates with $fm_{CYP2D6} < 75\%$ and 4–54 for substrates with $fm_{CYP2D6} > 75\%$ and CYP2D6-related UFs ranged for AUCs and clearances from 1.6 to 63.5 and 1.4–116 (95th centile) and 2.0–73.9 and 1.5–151.5 (97.5th centile). Such large inter-phenotypic differences between EMs and PMs highlight that for major CYP2D6 substrates ($fm_{CYP2D6} > 75\%$), the default TK UF would not be appropriate to cover inter-phenotypic differences either at the 95th or 97.5th centiles.

Figs. 2 and 3 illustrate meta-regressions which provide a quantitative basis to establish a relationship between the fm_{CYP2D6} (i.e. fraction of dose metabolised by CYP2D6 in EMs) and the inter-phenotypic differences in AUCs and Clearances for the Caucasian population. fm_{CYP2D6} for each CYP2D6 substrate are reported in Supplementary Materials. The meta-regressions were generated using the EFSA Benchmark dose modelling tool using the equations:

$$f < - \exp(\log(a) + \log(c) * (1 - \exp(-b * x^d)))$$

$$b < - \log(1 - (\log(CES + 1))/(\log(c)))/(CED^d)$$

with CED as critical effect dose while CES = 0.05 and c = 10⁴⁰ fixed values with CES as critical effect size set to 5%.

Results for the AUC and CL meta-regressions are provided in supplementary material 2 and expressed as loglikelihood values and Akaike Information Criteria, to characterise the relative goodness of fit as described in the EFSA guidance document on Benchmark dose modelling (EFSA Scientific Committee et al., 2017b).

Such meta-regression allows to integrate inter-phenotypic differences in PK for CYP2D6 probe substrates and can be implemented when isoform specific data are available for the hazard characterisation of CYP2D6 chemicals in food including regulated products (e.g. pesticides, food additives etc) and contaminants. The reader is referred to the discussion section for a full account on potential approaches to do so.

For markers of acute exposure (C_{max}), tables 5 and 6 illustrate CYP2D6-related UFs (calculated to cover 95% and 97.5% of EMs and PMs respectively). According to these results, CYP2D6-related UFs for the EM ranged from 1.1 to 9.5 (95th) and 1.1–14.4 (97.5th) whereas for PM Caucasians the range was larger [1.1–11 (95th) and 1.2–12.4 (97.5th)] respectively. Overall, the CYP2D6-related UFs were mostly below the default TK UF (3.16) to cover 97.5% of the population for substrates with $fm_{CYP2D6} < 75\%$. In contrast, for major CYP2D6 probe-substrates with $fm_{CYP2D6} > 75\%$, the default TK UF would not be appropriate to cover 95% of EM and PM populations.

This conclusion is further highlighted in Fig. 4 with meta-regressions depicting the relationships between fm_{CYP2D6} and inter-phenotypic differences in C_{max}.

Table 1

Inter-individual differences in area-under the plasma-concentration curve (AUC) (ng^h/mL/mg/kg) of CYP2D6 probe substrates for Caucasian Extensive Metabolisers after oral administration.

Chemical	Ns	n	CV	GM	UF95 [95% CI]	UF97.5 [97.5% CI]		
Atomoxetine	4	62	72	3497.8	2.9	[2.1–4.8]	3.6	[2.5–6.5]
Carvedilol	1	6	74	420.1	3.4	[1.5–555]	4.3	[1.7–1861]
Carvedilol (R)	1	6	87	436.5	3.5	[1.6–16.4]	4.3	[1.7–26.2]
Carvedilol (S)	1	6	57	167.5	2.4	[1.4–7.5]	2.8	[1.5–10.5]
Chlorpheniramine (R)	1	6	55	1135.9	2.3	[1.4–7.1]	2.7	[1.5–9.9]
Chlorpheniramine (S)	1	6	72	2623.9	2.9	[1.5–11.5]	3.5	[1.6–17.3]
Citalopram	2	35	7	3434.1	1.1	[1.1–1.2]	1.2	[1.1–1.3]
Desipramine	7	97	70	677.7	2.8	[2.2–4.1]	3.5	[2.6–5.3]
Dextromethorphan	2	10	92	203.4	4.0	[1.8–203]	5.2	[2.0–203]
Encainide	1	7	58	174.3	2.6	[1.4–58]	3.2	[1.5–127]
Fesoterodine	8	115	44	424.6	2.0	[1.7–2.5]	2.3	[1.9–3]
Fluoxetine	2	28	62	2804.8	2.6	[1.8–4.3]	3.1	[2–5.6]
Haloperidol	2	25	54	515.2	2.3	[1.6–5.3]	2.7	[1.8–7.3]
MDMA	2	27	31	1417.1	1.7	[1.4–2.5]	1.8	[1.4–3]
Metoprolol	11	116	68	544.1	2.8	[2.2–3.9]	3.3	[2.5–5]
Mirabegron	1	8	15	543.3	1.3	[1.1–2.7]	1.3	[1.1–3.2]
Mirtazapine	1	35	4	1520.4	1.1	[1.0–1.1]	1.1	[1–1.1]
Nebivolol	3	70	87	105.2	3.5	[2.5–5.9]	4.4	[2.9–8.3]
Nefazodone	2	30	51	1408.2	2.2	[1.6–4.1]	2.6	[1.8–5.3]
Nortriptyline	1	12	7	3209.9	1.1	[1.1–1.4]	1.2	[1.1–1.5]
Oxycodone	3	53	37	320.7	1.8	[1.5–2.5]	2.0	[1.6–3]
Paroxetine	1	9	69	367.1	2.8	[1.6–8.0]	3.4	[1.7–11.2]
Propafenone	2	12	76	489.9	3.2	[1.7–23.5]	4.0	[1.9–43.4]
Ramosetron	1	23	45	2314.3	2.1	[1.5–4.0]	2.4	[1.7–5.2]
Risperidone	4	78	109	961.8	4.3	[3.0–7.8]	5.8	[3.6–11.5]
Tramadol	2	14	31	1078.3	1.7	[1.3–3.6]	1.8	[1.3–4.6]
Tramadol (R)	5	66	38	903.7	1.8	[1.5–2.4]	2.1	[1.7–2.9]
Tramadol (S)	5	66	31	740.2	1.6	[1.4–2.1]	1.8	[1.5–2.4]
Venlafaxine	3	28	81	618.6	3.3	[2.0–8.7]	4.1	[2.3–13.1]

*Ns: number of studies, n: number of EM individuals, GM: geometric mean.

UF 95[95% CI]: CYP2D6-related Uncertainty factor to cover the 95th centile of the population and 95% confidence interval.

UF 97.5[95% CI]: CYP2D6-related Uncertainty factor to cover the 97.5th centile of the population and 95% confidence interval.

Table 2

Inter-individual differences in Clearance of CYP2D6 probe substrates (ml/min/kg bw) for Caucasian Extensive Metabolisers after oral administration.

Chemical	Ns	n	CV	GM	UF95 [95% CI]	UF97.5 [97.5% CI]		
Amitriptyline	1	4	82	0.64	3.3	[1.4–27.4]	4.1	[1.5–47.4]
Atomoxetine	3	36	78	0.62	3.2	[2.1–7.1]	3.9	[2.4–10.4]
Carvedilol (R)	1	10	57	0.07	2.5	[1.5–16.4]	3.0	[1.6–28.3]
Chlorpheniramine (R)	1	6	40	17.02	1.9	[1.3–4.5]	2.1	[1.3–5.8]
Chlorpheniramine (S)	1	6	46	7.84	2.0	[1.3–5.5]	2.3	[1.4–7.3]
Citalopram	2	35	6	5.66	1.1	[1.1–1.2]	1.1	[1.1–1.2]
Codeine	1	8	51	0.53	2.3	[1.4–21.8]	2.7	[1.5–39.2]
Desipramine	10	85	53	1.5	2.3	[1.8–3.2]	2.7	[2.0–4.0]
Dextromethorphan	1	3	221	11.45	9.5	[1.8–833]	14.2	[2.0–2609]
Duloxetine	1	12	33	14.72	1.7	[1.3–4.3]	1.9	[1.4–5.6]
Encainide	1	13	79	1.52	3.3	[1.8–21.7]	4.2	[2.0–39.1]
Fesoterodine	1	8	55	30.27	2.4	[1.6–6.5]	2.8	[1.8–9.4]
Hydrocodone	1	6	44	0.18	2.2	[1.3–54.4]	2.5	[1.4–116]
Imipramine	1	6	34	2500	1.8	[1.2–23]	2.0	[1.3–42]
Metoprolol	10	124	75	24.73	3.0	[2.4–4.3]	3.7	[2.8–5.7]
Mexiletine	2	20	56	0.5	2.4	[1.6–6.0]	2.8	[1.8–8.4]
Mirabegron	1	8	15	30.98	1.3	[1.1–2.6]	1.4	[1.1–3.1]
Mirtazapine	1	35	5	10.97	1.1	[1.1–7.0]	1.1	[1.1–9.5]
Nebivolol	1	12	52	173.99	2.3	[1.3–13.6]	2.8	[1.4–22.6]
Nortriptyline	1	4	82	0.63	3.3	[1.4–27.9]	4.1	[1.5–47.7]
Oxycodone	1	7	37	35.95	1.8	[1.3–3.7]	2.0	[1.3–4.7]
Propafenone	6	104	76	10.43	3.0	[2.3–4.4]	3.8	[2.8–5.9]
Propranolol	1	6	24	0.18	1.5	[1.2–9.8]	1.6	[1.2–15.2]
Risperidone	3	91	72	15.03	2.9	[2.2–4.3]	3.6	[2.6–5.7]
Sparteine	1	16	53	0.27	2.3	[1.5–7.3]	2.6	[1.6–10.1]
Tolterodine	3	34	102	16.91	4.1	[2.4–11.4]	5.4	[2.9–18.1]
Tramadol (R)	3	50	68	8.2	2.7	[2.0–4.1]	3.3	[2.3–5.3]
Tramadol (S)	3	50	64	10.41	2.7	[2–4.6.0]	3.2	[2.2–6.1]
Venlafaxine	6	51	73	26.4	2.9	[2.1–4.4]	3.6	[2.4–5.8]

*Ns: number of studies, n: number of EM or PM individuals, GM: Geometric Mean.

UF 95[95% CI]: CYP2D6-related Uncertainty factor to cover the 95th centile of the population and 95% confidence interval.

UF 97.5[95% CI]: CYP2D6-related Uncertainty factor to cover the 97.5th centile of the population and 95% confidence interval.

Table 3

Inter-individual differences in area-under the plasma-concentration curve (AUC) (ng^{*}h/mL/mg/kg) of CYP2D6 probe substrates for Caucasian Poor Metabolisers and inter-phenotypic differences compared to Caucasian Extensive Metabolisers after oral administration.

Chemical	Ns	n	CV	GM	Ratio GM	UF95 [95% CI]	UF97.5 [95% CI]		
Carvedilol	1	6	55	4332.3	10.3	63.5	[2.1–2460]	73.9	[2.4–5253]
Carvedilol (R)	1	6	40	1235.8	2.8	5.4	[2.3–15.7]	6.1	[2.5–19]
Carvedilol (s)	1	6	43	333.5	2.0	3.9	[1.6–12.4]	4.5	[1.8–15.6]
Dextromethorphan	1	2	10	530.9	2.6	3.1	[1.4–10.7]	3.3	[1.5–12.3]
Fesoterodine	8	58	38	797.5	1.9	3.5	[2.5–5.2]	3.9	[2.8–6.2]
Fluoxetine	1	10	20	6372.7	2.3	3.3	[1.8–8.4]	3.5	[1.9–10]
Haloperidol	1	8	80	265.2	0.5	1.6	[0.6–6.5]	2.0	[0.6–9.4]
Metoprolol	4	17	24	2201	4.0	6.0	[3.9–9.5]	6.5	[4.2–10.6]
Mirabegron	1	8	32	631.5	1.2	2.0	[1–10.9]	2.3	[1.0–15.3]
Mirtazapine	1	9	8	2049.5	1.3	1.6	[0.9–3.0]	1.6	[1.0–3.2]
Nebivolol	3	8	20	3210.9	30.5	44	[25.7–140]	47	[27–177]
Oxycodone	1	10	63	134.9	0.4	1.1	[0.4–3.2]	1.3	[0.5–4.2]
Paroxetine	1	8	61	2718.1	7.4	18.7	[7.3–60.2]	22.1	[8.2–79.8]
Propafenone	2	12	30	4491.8	9.2	15.4	[8.5–42]	16.9	[9.1–54.3]
Ramosetron	1	8	43	2584.8	1.1	2.4	[1.0–20.9]	2.7	[1.1–33]
Risperidone	3	14	26	9122.5	9.5	14.8	[9–33.4]	16	[9.6–40]
Tramadol	1	7	33	2046	1.9	3.3	[1.5–7.9]	3.6	[1.7–9.4]
Tramadol (R)	5	24	48	1831.3	2	4.4	[2.5–10.7]	5.1	[2.8–14.1]
Tramadol (S)	5	24	43	1324.6	1.8	3.6	[2.1–8.0]	4.1	[2.3–10.2]
Venlafaxine	3	20	66	2986	4.8	13.6	[6.7–48]	16.5	[7.7–71.3]

*Ns: number of studies, n: number of EM or PM individuals, GM: Geometric mean, Ratio GM: geometric mean ratio between PMs and EMs, UF 95[95% CI]: CYP2D6-related Uncertainty factor to cover the 95th centile of the population and 95% confidence interval. UF 97.5[95% CI]: CYP2D6-related Uncertainty factor to cover the 97.5th centile of the population and 95% confidence interval.

Table 4

Inter-individual differences in Clearance of CYP2D6 probe substrates (ml/min/kg bw) of CYP2D6 substrates for Caucasian Poor Metabolisers and inter-phenotypic differences compared to Caucasian Extensive Metabolisers after oral administration.

Chemical	Ns	n	CV	GM	Ratio GM	UF95 [95% CI]	UF97.5 [95% CI]		
Amitriptyline	1	3	17	0.3	2.1	6.9	[0.3–174]	8.7	[0.3–222]
Codeine	1	6	44	0.52	1.0	2.2	[0.1–57.6]	2.6	[0.1–66.8]
Desipramine	4	22	34	0.21	7	15.8	[9.0–27.9]	18.5	[10.5–33]
Encainide	1	4	39	0.06	26	64.3	[2.9–475]	79.9	[3.6–591]
Fesoterodine	2	10	36	18.28	1.7	3.8	[0.4–34.6]	4.5	[0.5–40.7]
Hydrocodone	1	5	25	0.11	1.7	3.3	[0.1–80.6]	3.8	[0.2–92.2]
Imipramine	1	6	24	1400	1.8	1.4	[0.1–13.9]	1.5	[0.1–15.6]
Metoprolol	8	53	36	4.19	5.9	17.2	[5.7–40]	21.3	[7–49.8]
Mexiletine	1	4	71	0.28	1.8	4.1	[0.4–13.3]	4.8	[0.5–15.6]
Mirabegron	1	8	32	25.95	1.2	1.5	[0.1–33.9]	1.6	[0.1–35]
Mirtazapine	1	9	8	8.23	1.3	1.4	[0.1–30]	1.5	[0.1–30.5]
Nebivolol	1	3	21	3.23	53.9	95.5	[3.5–686]	111.6	[4.1–803]
Nortriptyline	1	4	14	0.3	2.1	6.7	[0.3–154]	8.5	[0.4–193]
Propafenone	1	13	30	1.76	5.9	17.9	[3.8–80.8]	22.1	[4.6–99.8]
Propranolol	1	6	24	0.08	2.1	3.0	[0.1–67.7]	3.2	[0.2–72.8]
Risperidone	2	11	36	1.58	9.5	24.3	[3.0–104.5]	29.8	[3.6–128]
Sparteine	1	14	38	0.04	6.2	11.2	[0.6–76.4]	13.2	[0.7–89.8]
Tolterodine	3	24	43	0.54	31.4	116	[19.8–409]	151.5	[25.9–537]
Tramadol (R)	3	14	54	4.25	1.9	5.3	[0.8–33.9]	6.4	[1.0–40.9]
Tramadol (S)	3	14	46	8.8	1.2	3.1	[0.5–19.3]	3.7	[0.6–23.3]
Venlafaxine	6	40	46	5.82	4.5	13.1	[3.7–47.6]	16.1	[4.5–58.9]

*Ns: number of studies, n: number of EM or PM individuals, GM: geometric mean; Ratio GM: geometric mean ratio PM/EM, UF 95[95% CI]: CYP2D6-related Uncertainty factor to cover the 95th centile of the population and 95% confidence interval. UF 97.5[95% CI]: CYP2D6-related Uncertainty factor to cover the 97.5th centile of the population and 95% confidence interval.

3.2.2. Inter-phenotypic differences for CYP2D6 metabolism in Asian populations

Variability in AUC and clearance for chronic exposure in Asian EMs are presented in table 7 and 8 and ranged from 22 to 77% (AUC) and 28–91% (clearance). Since almost no information was available for $f_{mCYP2D6}$ in Asian PMs, f_m values were calculated using the data for the Caucasian population. CYP2D6-related UFs were similar for the AUC and Clearance from 1.4 to 3.1 and 1.6–3.6 (95th centile) and 1.4 to 3.9 and 1.8–4.5 (97.5th centile) and were mostly within the default TK UF except for a few major CYP2D6 substrates ($f_{mCYP2D6} > 75\%$).

Table 9 and table 10 illustrate the analysis of inter-phenotypic differences between Asian EMs and IMs with differences in internal dose ranging from 1 to 4 (AUC, clearance) considering all substrates. Such differences were much lower compared to those from Caucasian EMs

and PMs which is consistent with the very low frequency of PMs in Asian populations (<1%) and the low frequency of IMs in Caucasian populations. CYP2D6-related UFs for Asian IMs ranged from 2.5 to 11 (95th centile) and 3 to 14 (97.5th centile) for markers of chronic exposure (AUC and Clearance) highlighting that for major CYP2D6 substrates ($f_m > 75\%$), the default TK UF would not cover inter-phenotypic differences in the Asian population. This finding is consistent with the activity of allele CYP2D6*10, responsible for the IM phenotype in Asian populations, which is associated with a reduced but functional CYP2D6 activity.

Fig. 4 illustrates the meta-regression between $f_{mCYP2D6}$ and inter-phenotypic differences in EM and IM Asian populations. As highlighted above, differences between Asian EM and IM phenotypes were much smaller compared to those between Caucasian EM and PM

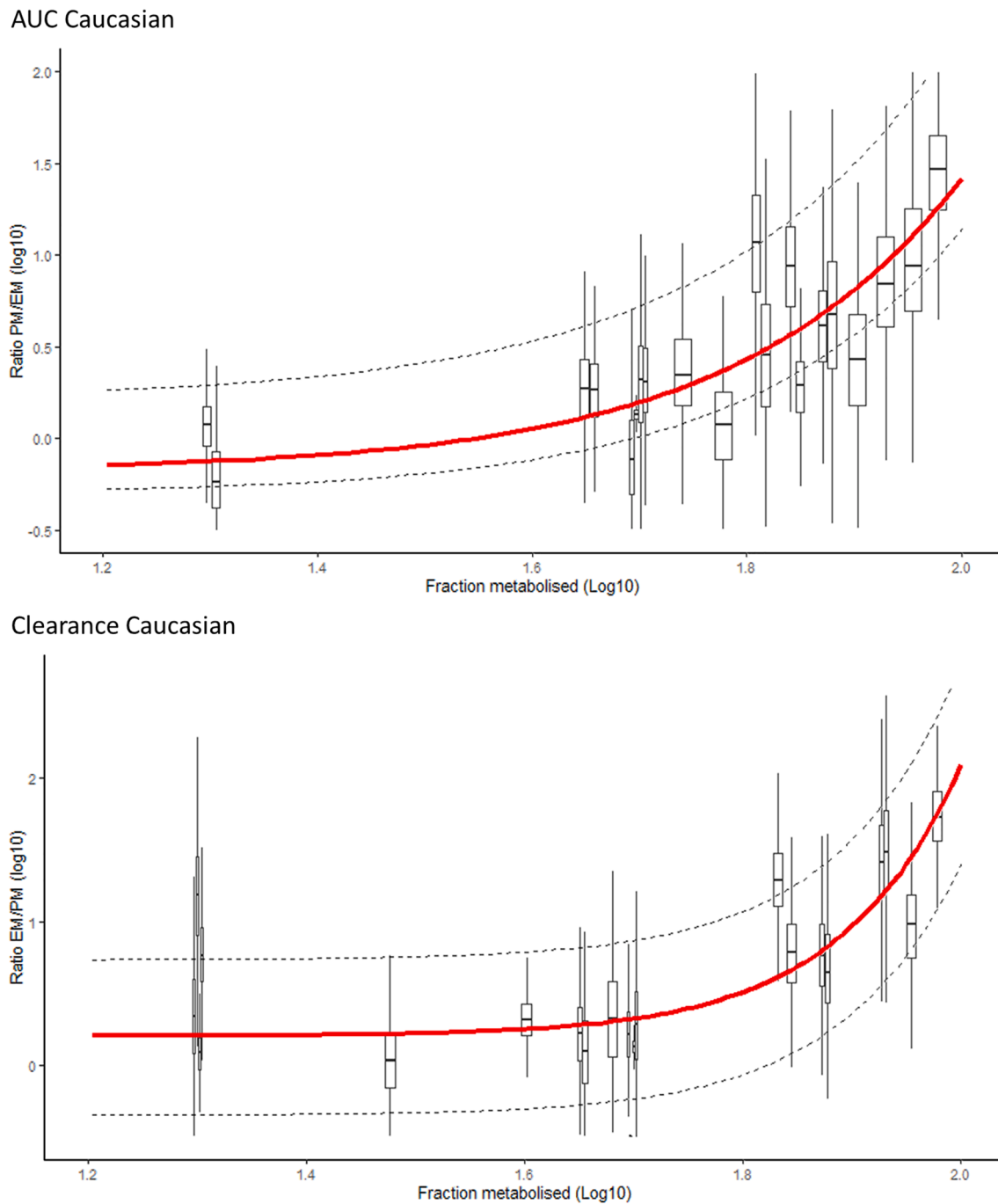


Fig. 2. Relationship between inter-phenotypic differences (EM and PM) and extent of CYP2D6 metabolism (EMs) in Caucasian populations for markers of Chronic exposure (area-under the plasma-concentration curve (AUC) and Clearance). (red line: median, dashed lines: IC95%). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

phenotypes.

Table 11 and table 12 present CYP2D6-related UFs for C_{max} as a marker of acute exposure in EM Asian populations and IMs (inter-phenotypic differences between EMs and IMs) respectively. Overall, variability in EM Asians ranged from 6 to 177% and CYP2D6-related UFs from 1.1 to 5.1 (95th) and 1.1–6.9 (97.5th). For the IM subgroup, variability in C_{max} ranged from 13 to 128% with CYP2D6-related UFs for Asian IMs ranging from 1.2 to 9.0 (95th centile) and 1.3 to 11.2 (97.5th centile). These results highlight that for major CYP2D6 substrates ($f_m > 75\%$), the default TK UF would not be cover IMs.

Fig. 5 illustrates the meta-regression between $f_{m_{CYP2D6}}$ and inter-phenotypic differences in EM and IM Asian populations for C_{max} . As

highlighted above, differences between these two phenotypes were smaller compared to those between Caucasian EM and PM populations. These CYP2D6-related distributions in Asian populations can be implemented to integrate inter-phenotypic differences in CYP2D6 metabolism for the hazard characterisation of food-related chemicals and allow to replace the default TK UF, in situations under which, it can be identified as inadequate.

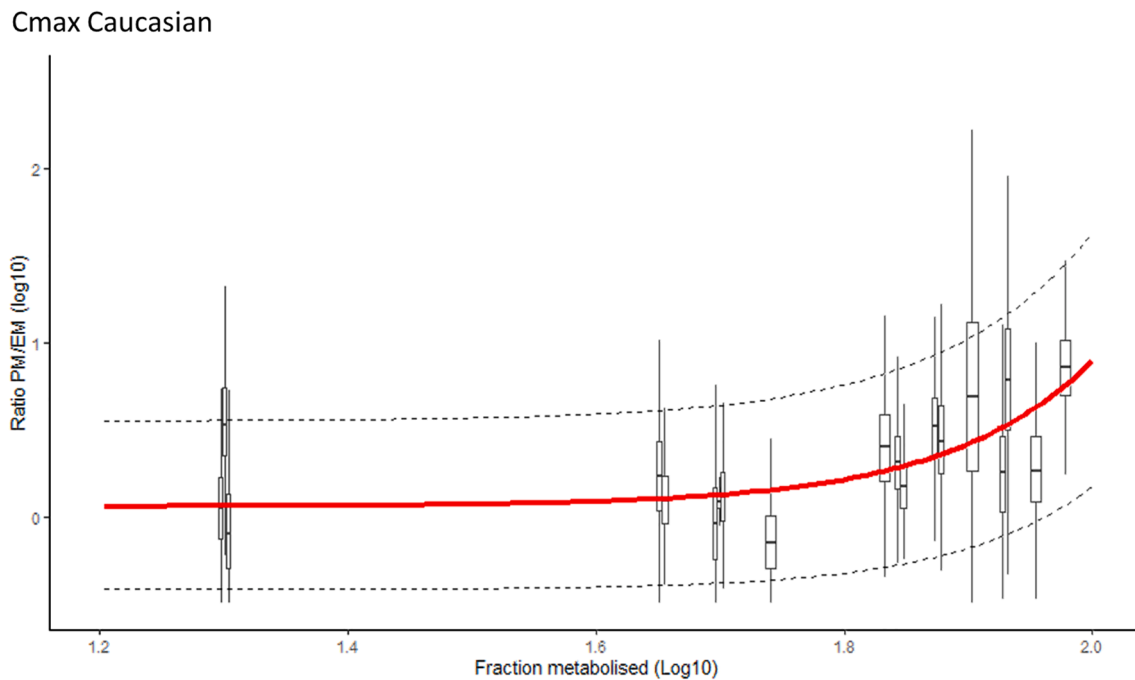


Fig. 3. Relationship between inter-phenotypic differences (EM and PM) and extent of CYP2D6 metabolism (EMs) in Caucasian populations for markers of acute exposure as peak plasma concentration (Cmax). (red line: median, dashed lines: IC95%). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 5

Inter-individual differences in peak plasma concentration (Cmax) (ng*h/mL/mg/kg) of CYP2D6 substrates for Caucasian Extensive Metabolisers after oral administration.

Chemical	Ns	n	CV	UF95 [95% CI]	UF97.5 [97.5% CI]		
Atomoxetine	5	76	91	3.6	[2.6–6.2]	4.6	[3.1–8.8]
Chlorpheniramine (R)	1	6	24	1.5	[1.1–7.6]	1.6	[1.2–11.2]
Chlorpheniramine (S)	1	6	35	1.7	[1.2–3.9]	1.9	[1.3–4.9]
Citalopram	2	35	7	1.1	[1.1–1.2]	1.1	[1.1–1.2]
Desipramine	15	172	70	2.8	[2.3–3.7]	3.4	[2.7–4.7]
Dextromethorphan	2	10	226	9.5	[2.5–141.9]	14.4	[3–141.9]
Duloxetine	1	12	112	4.8	[2.1–66.3]	6.5	[2.4–148]
Encainide	1	5	76	3.1	[1.5–16.4]	3.7	[1.6–26.2]
Fesoterodine	8	115	43	2.0	[1.7–2.5]	2.3	[1.9–2.9]
Fluoxetine	2	28	48	2.1	[1.6–4.1]	2.5	[1.7–5.4]
Haloperidol	2	25	49	2.2	[1.6–4.3]	2.5	[1.7–5.7]
Imipramine	3	18	94	3.8	[2.1–18]	5.0	[2.4–31.7]
MDMA	2	27	31	1.6	[1.4–2.5]	1.8	[1.4–3.0]
Metoprolol	14	164	54	2.3	[2–2.9]	2.7	[2.2–3.5]
Mirabegron	1	8	28	1.6	[1.2–5.8]	1.7	[1.3–8.3]
Mirtazapine	1	35	5	1.1	[1.1–1.1]	1.1	[1.1–1.2]
Nebivolol	3	70	49	2.2	[1.7–3]	2.5	[1.9–3.7]
Nefazodone	2	30	219	9.5	[3.9–63.4]	14.7	[5.1–141]
Oxycodone	4	60	29	1.6	[1.4–2]	1.8	[1.5–2.3]
Paroxetine	1	9	45	2.1	[1.4–12]	2.4	[1.4–19.1]
Propafenone	3	32	45	2.1	[1.6–3.5]	2.4	[1.7–4.4]
Reboxetine	2	23	45	2.1	[1.5–4]	2.4	[1.6–5.3]
Risperidone	4	84	71	2.9	[2.2–4.3]	3.5	[2.5–5.7]
Tolterodine	3	22	113	4.7	[2.4–21]	6.2	[2.8–37.7]
Tramadol	2	14	27	1.6	[1.2–3.1]	1.7	[1.3–3.8]
Tramadol (R)	4	58	30	1.6	[1.4–2.1]	1.8	[1.5–2.4]
Tramadol (S)	4	58	27	1.6	[1.4–1.9]	1.7	[1.4–2.2]
Venlafaxine	3	28	56	2.4	[1.7–4.9]	2.8	[1.9–6.7]

*Ns: number of studies, n: number of EM or PM individuals, ratio GM: geometric mean ratio PM/EM,

UF 95[95% CI]: CYP2D6-related Uncertainty factor to cover the 95th centile of the population and 95% confidence interval.

UF 97.5[95% CI]: CYP2D6-related Uncertainty factor to cover the 97.5th centile of the population and 95% confidence interval.

Table 6

Inter-individual differences in peak plasma concentration (C_{max}) (ng^{*}h/mL/mg/kg of CYP2D6 substrates (ml/min/kg bw) of CYP2D6 substrates for Caucasian Poor Metabolisers and inter-phenotypic differences compared to Caucasian Extensive Metabolisers after oral administration.

Chemical	Ns	n	CV	GM	Ratio GM	UF95 [95% CI]	UF97.5 [95% CI]
Atomoxetine	1	2	4	1030.6	1.9	2.1	[1.0–5.3]
Desipramine	2	16	22	57.4	2.5	3.6	[2.1–7.5]
Dextromethorphan	1	2	1	59.8	4.6	4.7	[2.4–9.1]
Fesoterodine	8	58	53	60	1.7	3.9	[2.6–6.7]
Fluoxetine	1	10	26	74.1	0.7	1.1	[0.5–3.5]
Haloperidol	1	8	68	19.5	0.8	2.2	[0.7–7.8]
Metoprolol	7	41	27	301.9	3.2	5	[3.4–7.6]
Mirabegron	1	8	51	105.8	1.1	2.4	[0.90–7.1]
Mirtazapine	1	9	11	39	1.2	1.5	[0.70–3.4]
Nebivolol	2	6	27	140.5	7.1	11.1	[5.2–28]
Oxycodone	1	10	61	434.9	4.2	10.1	[1.7–57]
Paroxetine	1	8	60	104.5	1.8	4.4	[1.5–14.2]
Propafenone	1	6	23	345.7	2.0	3	[1.7–7.5]
Reboxetine	1	3	71	1802.3	0.6	1.9	[0.4–27.4]
Risperidone	3	14	26	650.1	1.9	2.9	[1.7–6.9]
Tolterodine	4	24	36	559.4	6.1	11.1	[6.3–19.1]
Tramadol	1	7	28	126.3	1.5	2.5	[1.0–15.5]
Tramadol (R)	4	22	34	145.3	1.3	2.4	[1.3–5.4]
Tramadol (S)	4	22	36	120.5	1.3	2.3	[1.3–5.1]
Venlafaxine	3	20	37	131.3	2.7	5	[2.8–12]

*Ns: number of studies, n: number of EM or PM individuals, ratio GM: geometric mean ratio PM/EM,

UF 95[95% CI]: CYP2D6-related Uncertainty factor to cover the 95th centile of the population and 95% confidence interval.

UF 97.5[95% CI]: CYP2D6-related Uncertainty factor to cover the 97.5th centile of the population and 95% confidence interval.

4. Conclusion: Implications for risk assessment of chemicals in food and emerging designer drugs

4.1. Inter-phenotypic differences in CYP2D6 between Caucasian and Asian populations

The polymorphic CYP2D6 isoform is expressed in the human brain and liver. The isoform is involved in xenobiotic metabolism including over 25% of known pharmaceuticals, a wide range of chemicals relevant to food safety and a growing number of emerging designer drugs of public health concern. In addition, there is growing evidence that CYP2D6 plays a role in the metabolism of endogenous compounds, such as neurotransmitters. From the 133 star alleles known to date, four major broad human phenotypes have been classified using activity scores (UMs, EMs, IMs and PMs) and their frequencies vary considerably across world populations.

Here, inter-phenotypic differences in CYP2D6 metabolism for Caucasian and Asian populations have been quantified by means of extensive literature searches and Bayesian meta-analyses from a large database reporting PK data for minor and major CYP2D6 substrates on markers of chronic oral exposure (AUC and clearance) and acute (C_{max}) exposure. The computed kinetic parameters resulted in the largest open source database, quantifying inter-phenotypic differences for the kinetics of CYP2D6 probe substrates in Caucasian and Asian populations, to date. The database is available in supplementary material (CYPD6 DB) and EFSA knowledge junction (DOI to added). The hierarchical Bayesian model provided a means to quantify the impact of sample sizes and heterogeneity within studies, as well as inter-study and inter-substrate variability. Inter-phenotypic differences between EMs and PMs in Caucasian populations were shown to be larger compared to those in Asian populations. Indeed, the two main phenotypes in Asian populations are EMs and IMs since PMs have very low frequencies (<1%) and consequently, data were very scarce for the later.

Overall, inter-individual variability after oral exposure for major CYP2D6 substrates was also larger in Caucasian EMs compared to that in Asian EMs (CV ≥ 50% vs 40%). Inter-phenotypic differences between Caucasian EMs and PMs for major CYP2D6 probe substrates ($f_{mCYP2D6} > 75\%$) were above the current default TK UF particularly for PMs. Such inter-phenotypic differences between Asian EMs and IMs were also slightly above the TK UF but to a more minor extent compared to those

in Caucasian PMs. In Caucasian populations, the large inter-phenotypic differences have been hypothesised to be due to the presence of multiple copies of the CYP2D6 genes across Caucasian phenotypes (UM, EM, IM, PM) ranging from 0 copies to 13 copies of the gene and individuals with more than two functional CYP2D6 copies being considered as UMs (Dorne et al., 2002; Taylor et al., 2020). The most common haplotypes include CYP2D6*1, *2 and *4 in which duplications or multiplications are observed and are denoted as ‘CYP2D6*1xN’ ‘*2xN’ and ‘*4xN’ respectively. In addition, other rarer duplications have been identified including CYP2D6*6xN, *10xN, *17xN, *36xN, *41xN, *43xN and *45xN (Taylor et al., 2020). CYP2D6*4 allele is the most frequent haplotype leading to the PM phenotype in Caucasian populations whereas CYP2D6*10 is responsible for a decreased function in Asian populations (IM phenotype). Such polymorphisms have been hypothesised to be the likely outcome of the introgression of a Neanderthal genetic variant, since Neanderthal have been shown to be CYP2D6 PMs. This occurrence of such an introgression has been hypothesised involve admixture between “archaic” Neanderthals and modern humans so that 1–4% of the Neanderthal genome is present in the genome of modern humans in Europe. East Asian populations have been shown to have an even higher frequency of Neanderthal genes as the result of multiple interbreeding episodes (Chen et al., 2020; Villanea and Schraiber, 2019). In African populations, ancestral Neanderthal genes have been also shown to be present recently but such introgression has been hypothesised to be rare (Chen et al., 2020). To date the relationships between CYP2D6*4, CYP2D6*10, neanderthal genome and modern human genome across world populations still needs to be investigated to provide a rationale for differences in CYP2D6 phenotype frequencies and inter-phenotypic differences in CYP2D6 metabolism (Ingelman-Sundberg et al., 2014).

4.2. Meta-regression modelling and application for the risk assessment of food chemicals and emerging drugs of public health concern

From the overall body of evidence presented here, exponential meta-regressions between $f_{mCYP2D6}$ in EMs and inter-phenotypic differences (and CYP2D6-related UFs) have also been shown to differ between Caucasian and Asian populations. Such meta-regressions provide a potential quantitative tool to predict inter-phenotypic differences in the PK of CYP2D6 substrates for these populations. It is important to note that

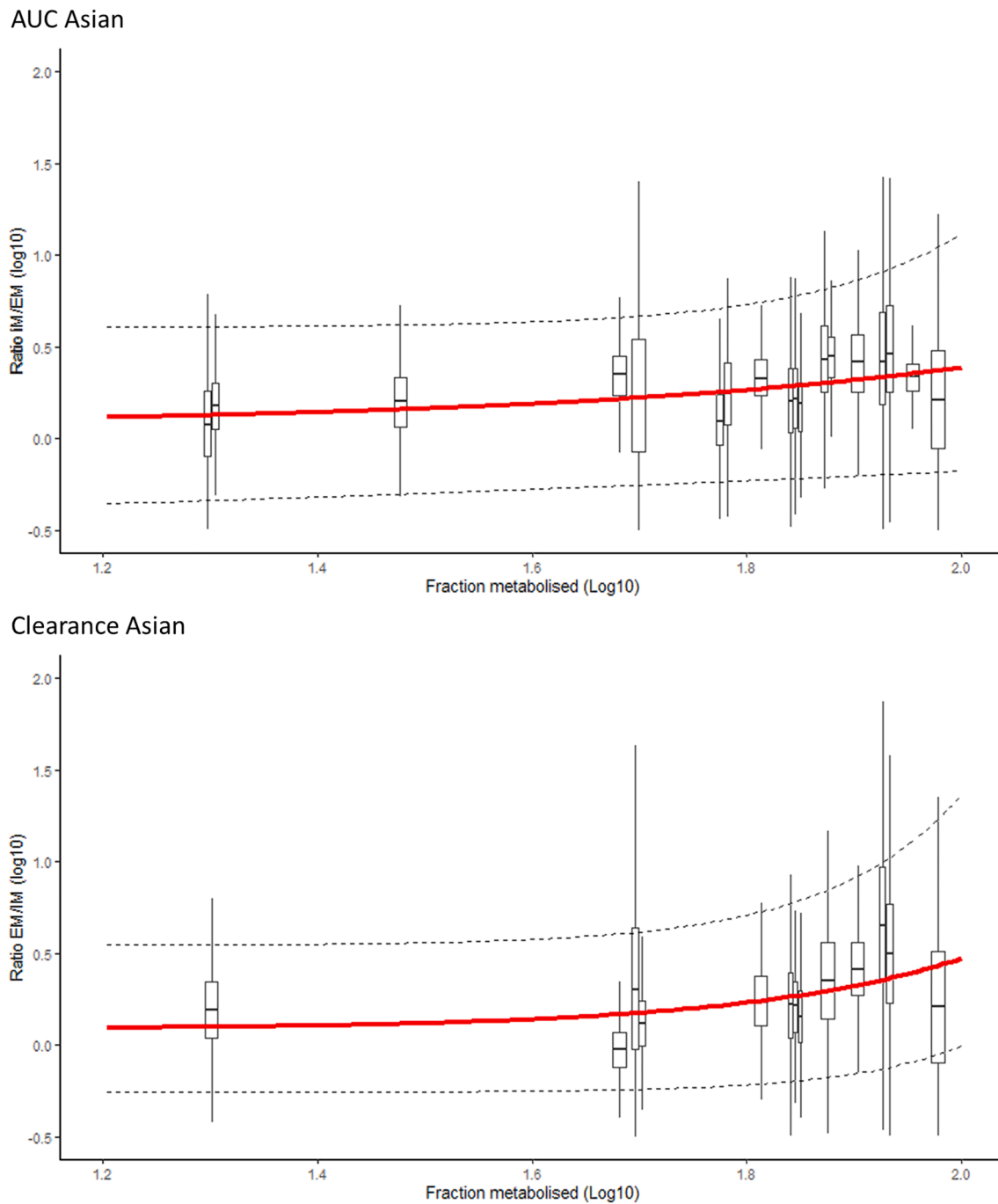


Fig. 4. Relationship between inter-phenotypic differences (EM and IM) and extent of CYP2D6 metabolism (EMs) in Asian populations for markers of Chronic exposure (area-under the plasma-concentration curve (AUC) and Clearance). (red line: median, dashed lines: IC95%). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

from the present analysis, CYP2D6 is assumed to be a detoxification metabolic pathway for chemicals. Consequently, PMs, and to a certain extent IMs, would be the most susceptible phenotypes and an increase in internal dose would result in an increase in the risk of adverse effects. In contrast, assuming that the toxicant is a bioactivated CYP2D6-mediated metabolite, PMs would have lower internal doses of the toxicant and, in contrast, EMs and UMs would produce higher concentrations of the toxic metabolites and would be the most susceptible group. For UMs, limited PK data are available and around 2-fold differences in internal dose were shown between UMs and EMs (Gan et al., 2007; Gassó et al., 2013; Kirchheiner et al., 2008; Samer et al., 2010). To conclude, assumptions on the consequence of metabolism (detoxification or bioactivation) are

important to implement CYP2D6-related variability distributions and CYP2D6-related UFs for the hazard assessment of chemicals. Relevant examples of CYP2D6 food chemicals and emerging designer drugs of public health concern are discussed below.

Relevant CYP2D6 substrates to the food safety area include diazinon and chlorpyrifos which are bioactivated to the oxon forms as potent inhibitors of acetylcholinesterase, as well as contaminants such as 4-nonyl-phenol, pyrrolizidine alkaloids to pyrrolic esters into liver carcinogens, 4-Ipomeanol as a pulmonary pre-toxin isolated from sweet potatoes, sparteine in lupin (Haduch and Daniel, 2018; Tang et al., 2001; Yang et al., 2019). In addition, fipronil and its metabolite fipronil sulfone have been shown to be strong competitive inhibitors of CYP2D6

Table 7

Inter-individual differences in area-under the plasma-concentration curve (AUC) (ng^{*}h/mL/mg/kg) of CYP2D6 substrates for Asian Extensive Metabolisers after oral administration.

Chemical	Ns	n	CV	GM	UF95 [95% CI]		UF97.5 [97.5% CI]	
Aripiprazole	6	39	24	13379.5	1.5	[1.3–1.9]	1.6	[1.3–2.1]
Atomoxetine	4	75	41	1992.3	1.9	[1.6–2.6]	2.2	[1.8–3.1]
Bromperidol	1	7	45	695.4	2.1	[1.3–25.7]	2.5	[1.4–46.8]
Carvedilol (R)	1	9	28	511.4	1.6	[1.2–5.1]	1.8	[1.3–6.9]
Carvedilol (S)	1	9	19	235.9	1.4	[1.1–2.9]	1.5	[1.2–3.6]
Citalopram	2	8	18	4437.1	1.4	[1.1–3.3]	1.4	[1.1–4.2]
Desipramine	1	7	50	712.7	2.3	[1.4–36.2]	2.7	[1.4–72.3]
Flecanaide	2	14	31	2007.2	1.7	[1.3–3.6]	1.9	[1.4–4.5]
Haloperidol	1	8	62	284.4	2.5	[1.5–7.3]	3.0	[1.6–9.9]
Lovastatine Lactone	2	11	44	30.2	2.0	[1.4–8.1]	2.4	[1.5–12.1]
Metoprolol	4	25	52	530.7	2.3	[1.6–4.9]	2.7	[1.8–6.6]
Mexiletine	1	5	25	1623.7	1.5	[1.2–3.1]	1.6	[1.2–3.6]
Nebivolol	1	12	77	83.6	3.0	[1.7–7.8]	3.8	[1.9–10.8]
Nortriptyline	4	20	30	1095.5	1.6	[1.3–2.8]	1.8	[1.4–3.4]
Paroxetine	3	25	77	202.5	3.1	[1.9–8.9]	3.9	[2.2–13.5]
Propafenone (R)	3	18	46	205.5	2.1	[1.5–5.2]	2.4	[1.6–7.1]
Propafenone (S)	3	18	38	284.8	1.9	[1.4–3.9]	2.1	[1.5–5.1]
Risperidone	5	64	22	2008.6	1.4	[1.3–1.7]	1.5	[1.4–1.9]
Tolterodine	3	44	73	163.2	3.0	[2.1–5.6]	3.7	[2.4–7.8]
Tramadol	5	50	31	2430.5	1.7	[1.4–2.2]	1.8	[1.5–2.5]
Venlafaxine	5	167	34	290	1.7	[1.5–2]	1.9	[1.7–2.2]

*Ns: number of studies, n: number of EM Asian individuals, GM: Geometric mean, UF 95 [95% CI]: CYP2D6-related Uncertainty factor to cover the 95th centile of the population and 95% confidence interval. UF 97.5 [95% CI]: CYP2D6-related Uncertainty factor to cover the 97.5th centile of the population and 95% confidence interval.

Table 8

Inter-individual differences in Clearance (mL/hr/kg) of CYP2D6 probe substrates for Asian Extensive Metabolisers after oral administration.

Chemical	Ns	n	CV	GM	UF95 [95% CI]		UF97.5 [97.5% CI]	
Atomoxetine	4	75	33	7.88	1.7	[1.5–2.1]	1.9	[1.6–2.5]
Haloperidol	1	8	91	22.07	3.6	[1.7–13.3]	4.5	[1.9–20.3]
Metoprolol	2	12	68	36.67	2.7	[1.6–6.5]	3.3	[1.8–9.2]
Mexiletine	1	5	28	10.34	1.6	[1.2–3.4]	1.7	[1.2–4.1]
Nebivolol	1	12	66	206.75	2.7	[1.6–5.9]	3.2	[1.8–8.3]
Nortriptyline	7	32	29	184.14	1.6	[1.3–3.4]	1.8	[1.4–4.3]
Paroxetine	2	15	72	117.82	2.9	[1.7–6.3]	3.5	[1.9–8.9]
Propafenone (R)	3	18	52	35.6	2.3	[1.5–6.5]	2.7	[1.7–9.3]
Propafenone (S)	3	18	33	26.2	1.7	[1.3–3.3]	1.9	[1.4–4.1]
Carvedilol (R)	4	55	35	32.0	1.8	[1.5–2.4]	2.0	[1.6–2.8]
Carvedilol (S)	4	55	32	60.6	1.7	[1.4–2.2]	1.8	[1.5–2.5]
Tolterodine	2	28	67	6.36	2.8	[1.8–6.4]	3.4	[2.1–9.3]
Tramadol	7	139	37	6.27	1.8	[1.6–2.1]	2.0	[1.7–2.5]
Venlafaxine	1	23	37	20.2	1.8	[1.4–3.2]	2.0	[1.5–4]

*Ns: number of studies, n: number of EM Asian individuals, GM: Geometric mean, UF 95 [95% CI]: CYP2D6-related Uncertainty factor to cover the 95th centile of the population and 95% confidence interval. UF 97.5 [95% CI]: CYP2D6-related Uncertainty factor to cover the 97.5th centile of the population and 95% confidence interval.

(Carrão et al., 2019). Important considerations to apply CYP2D6-related variability distributions in a food safety context are human Exposure patterns to chemical residues since such exposure may most often be within a µg range (perhaps with the exception of some food additives) and the fact that such distributions were derived from data based on pharmaceutical exposure which are in the mg range. Hence, the CYP2D6-related variability distributions for inter-phenotypic differences can be considered as conservative tools and would need further calibration and testing using food-relevant exposure in humans for a range of chemicals.

Recent evidence highlighted that CYP2D6 may play a role in the endogenous metabolism of a number of substances and neurotransmitters in the brain but available data in humans are still insufficient to allow CYP2D6 phenotyping with endobiotics. Further work is required in this area to fully characterise the role of CYP2D6 in the metabolism of endogenous substrates and their relationship with the toxicity of xenobiotics particularly inhibitors of the isoform (Carrão et al., 2019; Magliocco et al., 2019).

The use of emerging designer drugs of public health concern is

rapidly increasing and because of their use as drugs of abuse and human exposures in the milligram range, the high variability in CYP2D6 metabolism and the associated neurotoxic and hepatic toxicity, adolescents and young adults constitutes a particularly sensitive subgroup. These include amphetamine-like designer drugs such MDMA and neurotoxins such as tetrahydroiso-quinolines and b-carbolines 1-methyl-4-phenyl-1,2,3,6-tetrahydro-pyridine (MPTP) and the stimulant designer drug methylone (methylenedioxy-methcathinone) (Pedersen et al., 2013; Haduch and Daniel, 2018). Methylenedioxy-derived designer drugs (MDD) of different drug classes such as aminoindanes, amphetamines, benzofurans, cathinones, piperazines, pyrrolidinophenones, and tryptamines have been shown to inhibit CYP2D6 *in vitro* with potencies in the range of clinically-relevant inhibitors (i.e. quinidine and fluoxetine) (Dinger et al., 2016). In addition, a number of phenethylamine-based designer drugs are also metabolised by CYP2D6 and CYP3A4 and N-acetylation (NAT1 and NAT2) such as 2C-EFLY, 2C-EFLY, and 2C-T-7-FLY and can induce psychostimulant and hallucinogenic effects (Wagmann et al., 2019). Since CYP2D6 is involved in the metabolism of endogenous substrates (serotonin, dopamine and others)

Table 9

Inter-individual differences in area-under the plasma-concentration curve (AUC) (ng³h/mL/mg/kg) of CYP2D6 substrates for Asian Intermediate Metabolisers and inter-phenotypic differences compared to Asian Extensive Metabolisers after oral administration.

Chemical	Ns	n	CV	GM	Ratio GM	UF95 [95% CI]	UF97.5 [95% CI]		
Aripiprazole	3	23	25	28387.3	2.1	3.2	[1.9–6.2]	3.5	[2.0–7.0]
Atomoxetine	2	25	41	5120.5	2.6	5.0	[2.6–12]	5.7	[2.8–14.7]
Bromperidol	1	7	42	804.5	1.2	2.5	[0.8–31.8]	2.9	[0.9–54.3]
Citalopram	1	4	37	6988.1	1.6	3.4	[1–3065]	3.9	[1.1–14055]
Flecanaide	1	7	34	2485.6	1.2	2.4	[0.8–19.1]	2.6	[0.9–30]
Haloperidol	1	7	108	374.9	1.3	5.6	[1.4–33.5]	7.3	[1.6–51.9]
Lovastatine Lactone	2	10	40	52.8	1.8	3.5	[1.5–17.9]	4.0	[1.6–26]
Metoprolol	2	8	33	1403.9	2.6	4.8	[2.1–27]	5.3	[2.3–38.3]
Mexiletine	2	10	38	2423.4	1.5	2.9	[1.3–13.5]	3.3	[1.4–19.1]
Nebivolol	1	12	82	119.2	1.4	4.7	[1.5–15.2]	5.9	[1.8–21.3]
Nortriptyline	1	5	21	2405.8	2.2	3.2	[1.3–8.2]	3.4	[1.3–9.2]
Paroxetine	3	20	68	560.4	2.8	8.0	[3.6–29.4]	9.8	[4.1–42.5]
Propafenone (R)	1	8	40	340.4	1.7	3.2	[1.2–8.7]	3.6	[1.3–10.5]
Propafenone (S)	1	8	41	474.8	1.7	3.2	[1.2–8.7]	3.6	[1.3–10.8]
Risperidone	2	41	12	4299.8	2.1	2.6	[1.5–4.5]	2.7	[1.6–4.7]
Tolterodine	3	26	55	506.5	3.1	7.4	[3.8–19.3]	8.8	[4.3–25.5]
Tramadol	2	20	34	3634.4	1.5	2.7	[1.4–6.3]	3.0	[1.5–7.6]
Venlafaxine	2	115	15	805.6	2.8	3.5	[2.3–5.7]	3.7	[2.4–6]

*Ns: number of studies, n: number of IM Asian individuals, GM: geometric mean, Ratio GM: geometric mean ratio IM and EM, UF 95[95% CI]: CYP2D6-related Uncertainty factor to cover the 95th centile of the population and 95% confidence interval. UF 97.5[95% CI]: CYP2D6-related Uncertainty factor to cover the 97.5th centile of the population and 95% confidence interval.

Table 10

Inter-individual differences in Clearance (mL/hr/kg) of CYP2D6 substrates for Asian Intermediate Metabolisers and inter-phenotypic differences compared to Asian Extensive Metabolisers after oral administration.

Chemical	Ns	n	CV	GM	Ratio GM	UF95 [95% CI]	UF97.5 [95% CI]		
Atomoxetine	2	25	38	2.98	2.6	4.4	[0.6–37.7]	4.8	[0.6–41.7]
Haloperidol	1	7	103	11.72	1.9	6.8	[0.2–54.8]	8.55	[0.2–69.9]
Metoprolol	1	3	34	16.34	2.2	5.1	[0.2–28.3]	6.2	[0.3–34.4]
Mexiletine	2	10	38	6.67	1.5	2.4	[0.3–21.6]	2.6	[0.3–23.6]
Nebivolol	1	12	97	148.71	1.4	3.2	[0.2–15]	3.9	[0.2–18.1]
Nortriptyline	2	8	10	192.99	1	1.3	[0.2–5.9]	1.5	[0.2–6.4]
Paroxetine	2	18	99	25.93	4.5	11.3	[1.4–43.1]	13.9	[1.7–52.7]
Propafenone (R)	1	8	35	21.13	1.7	3.7	[0.2–79.9]	4.3	[0.2–93.2]
Propafenone (S)	1	8	35	15.44	1.7	2.8	[0.1–59.5]	3.1	[0.2–66]
Carvedilol (R)	2	29	32	18.87	1.7	3.0	[0.4–24.3]	3.3	[0.4–27]
Carvedilol (S)	2	29	29	46.46	1.3	2.2	[0.3–17.8]	2.4	[0.3–19.5]
Tolterodine	1	3	93	1.84	3.5	1.6	[0.4–2.4]	9.8	[1.3–35.6]
Tramadol	3	65	35	4.37	1.4	2.6	[0.5–14.6]	2.9	[0.5–16.3]

*Ns: number of studies, n: number of IM Asian individuals, ratio GM: geometric mean ratio IM and EM Asians, UF 95[95% CI]: CYP2D6-related Uncertainty factor to cover the 95th centile of the population and 95% confidence interval. UF 97.5[95% CI]: CYP2D6-related Uncertainty factor to cover the 97.5th centile of the population and 95% confidence interval.

in the brain, investigating the consequences of CYP2D6 inhibition on the anabolism and metabolism of endogenous compounds by compounds such as MDD and its potential consequences on associated neurotoxicity would be of high relevance. Other relevant CYP2D6 substrate include fentanyl and related derivatives (e.g. butyrfentanyl, isofentanyl and furanoyl-1-benzyl-4-anilinopiperidine) which have been identified by the European monitoring centre for drugs and drug addiction (EMCDDA) as psychoactive substances of abuse and further characterisation of the role of CYP2D6 in their metabolism is recommended (Gampfer et al., 2020; Steuer et al., 2017; Wilde et al., 2019; Wu et al., 2015).

4.3. Integration of CYP2D6-related variability distributions in hazard characterisation

This manuscript has provided a basis to integrate CYP2D6-related variability distributions quantifying inter-phenotypic differences and uncertainty across Caucasian and Asian populations with isoform-specific *in vitro* data using assays from human cell lines (Bell et al., 2018; Blaauboer et al., 2012). This is a promising approach for developing quantitative *in vitro in vivo* extrapolation (QIVIVE) models and physiologically based kinetic (PBK) models and consequently reduce

uncertainty in chemical risk assessment as well as provide alternatives to animal testing. Such integration has been discussed recently for the Glucuronidation and Paraoxonase 1 metabolic pathways (Kasteel et al., 2020, Darney et al., 2020).

The derivation of chemical-specific adjustment factors quantifying CYP2D6-related variability for each phenotype and the whole population is feasible for specific compounds but would require the estimation of fm_{CYP2D6} in EMs as a basis to estimate inter-phenotypic differences. When data for the estimation of fm_{CYP2D6} in EMs are not available, assumptions can be formulated using expert judgement and knowledge on the ADME properties of the compound under assessment and these should be documented transparently using a weight of evidence (WoE) approach (EFSA Scientific Committee et al., 2017a). The database described here has also shown that major CYP2D6 substrates have high clearances which depends on CYP2D6 metabolism, liver blood flow and plasma protein binding as highlighted previously (Dorne et al., 2002; Chiba et al., 2012). Therefore, future predictions of chemical-specific variability and associated CYP2D6-related UFs, would require modelling relationships between quantitative CYP2D6 metabolism, liver blood flow and plasma protein binding in different world population and phenotypes. Typically, PMs would have lower clearances and liver blood flow may have a lower impact on inter-individual variability compared

Table 11Inter-individual differences in peak plasma concentration (C_{max}) (ng/mL/mg/kg) of CYP2D6 substrates for Asian Extensive Metabolisers after oral administration.

Chemical	Ns	n	CV	GM	UF95 [95% CI]	UF97.5 [97.5% CI]
Aripiprazole	6	39	24	316.4	1.5	[1.3–1.9]
Atomoxetine	4	75	31	441.8	1.7	[1.4–2.0]
Bromperidol	1	7	46	42.7	2.2	[1.3–26.4]
Citalopram	2	8	6	100.5	1.1	[1–1.5]
Codeine	2	10	25	88.1	1.5	[1.3–2.3]
Dehydroaripiprazole	2	13	117	18	5.1	[2.2–61.3]
Desipramine	1	7	7	1.2	1.1	[1–1.8]
Flecanaide	2	14	32	131.9	1.7	[1.3–3.7]
Haloperidol	1	8	46	17.9	2.1	[1.4–4.7]
Imipramine	1	7	80	19.1	3.2	[1.6–11.9]
Lovastatine lactone	2	11	61	3.7	2.6	[1.6–15.8]
Metoprolol	4	25	46	56.2	2.1	[1.5–4.3]
Mexiletine	1	5	12	140.1	1.2	[1.1–4.0]
Nebivolol	1	12	64	13.2	2.7	[1.6–14.9]
Nortriptyline	6	31	33	43	1.7	[1.4–2.8]
Paroxetine	3	25	61	11.7	2.6	[1.7–6.3]
Propafenone	1	16	87	81.3	3.6	[1.9–18.2]
Propafenone (R)	3	18	50	37.7	2.2	[1.5–5.6]
Propafenone (S)	3	18	44	54.3	2.0	[1.5–4.7]
Risperidone	4	55	16	314	1.3	[1.2–1.5]
Tolterodine	3	44	74	19.6	3	[2.1–6.0]
Tramadol	5	50	37	292.5	1.8	[1.5–2.5]
Venlafaxine	5	61	30	35.3	1.6	[1.5–1.8]

*Ns: number of studies, n: number of EM Asian individuals, GM: geometric mean, UF 95[95% CI]: CYP2D6-related Uncertainty factor to cover the 95th centile of the population and 95% confidence interval. UF 97.5[95% CI]: CYP2D6-related Uncertainty factor to cover the 97.5th centile of the population and 95% confidence interval.

Table 12Inter-individual differences in peak plasma concentration (C_{max}) (ng/mL/mg/kg) of CYP2D6 substrates for Asian Intermediate Metabolisers and inter-phenotypic differences compared to Asian Extensive Metabolisers after oral administration.

Chemical	Ns	n	CV	GM	Ratio GM	UF95 [95% CI]	UF97.5 [95% CI]
Aripiprazole	3	23	23	310.7	1.0	1.4	[0.9–2.6]
Atomoxetine	2	25	30	642.6	1.5	2.4	[1.4–4.7]
Bromperidol	1	6	13	41.1	1.0	1.2	[0.6–4.2]
Citalopram	1	4	63	94.8	0.9	2.5	[0.7–17.3]
Codeine	1	9	44	84.9	1.0	2.1	[0.8–14.1]
Dehydroaripiprazole	2	14	128	13.9	0.8	4.4	[1.3–64.1]
Flecanaide	1	7	27	144.1	1.1	1.8	[0.8–11.6]
Haloperidol	1	7	57	20.2	1.1	2.7	[1–9.7]
Lovastatine lactone	2	10	47	6.4	1.7	3.8	[1.7–24.2]
Metoprolol	2	8	62	127.1	2.3	5.8	[2–21]
Mexiletine	2	10	20	135.1	1.0	1.4	[0.8–3.6]
Nebivolol	1	12	51	14	1.1	2.5	[1–12.9]
Nortriptyline	2	9	52	69.1	1.6	3.9	[1.5–43.3]
Paroxetine	2	18	56	21.4	1.8	4.5	[2.2–14.9]
Propafenone (R)	1	8	21	57.1	1.5	2.2	[1–7.6]
Propafenone (S)	1	8	16	81	1.5	2.0	[1–6.1]
Risperidone	2	41	13	386.3	1.2	1.5	[1–2.5]
Tolterodine	1	8	76	56.2	2.9	9.0	[4.3–28.9]
Tramadol	2	20	45	369.8	1.3	2.6	[1.4–5]
Venlafaxine	3	21	20	76.6	2.2	3.1	[2–4.4]

*Ns: number of studies, n: number of IM individuals, Ratio GM: geometric mean ratio between IM and EM Asians.

UF 95[95% CI]: CYP2D6-related Uncertainty factor to cover the 95th centile of the population and 95% confidence interval.

UF 97.5[95% CI]: CYP2D6-related Uncertainty factor to cover the 97.5th centile of the population and 95% confidence interval.

to that in EMs since alternative metabolic routes to CYP2D6 in PMs often involve other major human CYP isoforms (i.e. CYP1A2, CYP3A4). It is important to note that the CYP2D6-specific distributions and UFs from this study, have been derived from pharmaceuticals and can be applied for compounds with relatively short half-lives (hours) and it remains to be seen if they would apply to compounds with longer half-lives (days, weeks, months). From a general perspective, a major data gap is the lack of human *in vivo* PK data and mechanistically validated *in vitro* assays in human intestinal, liver, and kidney cells. Further research and validation efforts in these areas would allow to characterise isoform-specific CYP metabolism and other isoforms from phase I and phase II enzymes as well as influx or efflux transporters for chemicals (Kasteel et al., 2020).

In addition, the current analysis has been performed on Caucasian and Asian populations and meta-analysis of PK parameters to depict inter-phenotypic differences for populations of other geographical origin could not be performed because of lack of PK data. Since Frequencies of different CYP2D6 phenotypes are rather different across world populations and, migration of specific populations is the rule rather than the exception in the modern world, further work is required to characterise such PK differences and develop CYP2D6-specific distributions for mixed populations to account for such heterogeneity.

In the light of these findings, it is recommended to further support the research community to investigate the characterisation of inter-phenotypic differences in CYP2D6 metabolism across world populations including age groups and polymorphic variants for which such

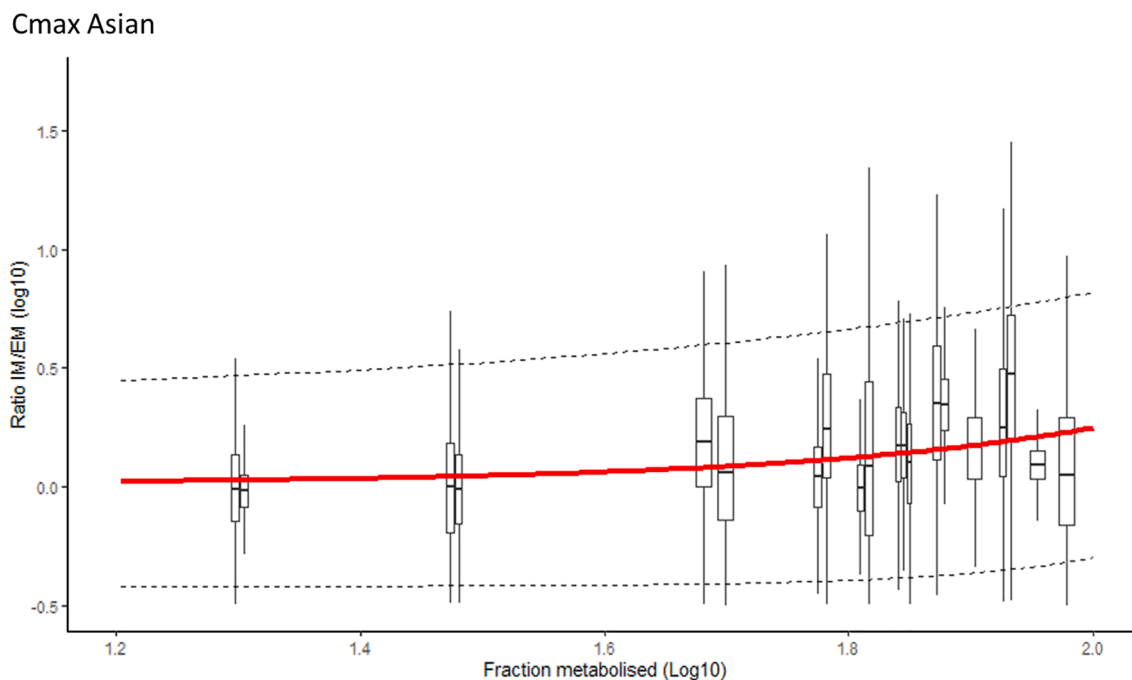


Fig. 5. Relationship between inter-phenotypic differences (EM and IM) and extent of CYP2D6 metabolism (EMs) in Asian populations for markers of acute exposure as peak plasma concentration (Cmax). (red line: median, dashed lines: IC95%). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

data are not available (African populations etc.). As discussed above, it is recommended to integrate isoform-specific *in vitro* metabolic information for food chemicals and emerging designer drugs into generic QIVIVE and PBK models using CYP2D6-related variability distributions and variability distributions from other isoforms using cases studies for routine implementation of these approaches into 21st century chemical risk assessment.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

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References

- Ahmed, A.T., Biernacka, J.M., Jenkins, G., Rush, A.J., Shinozaki, G., Veldic, M., Kung, S., Bobo, W.V., Hall-Flavin, D.K., Weinshilboum, R.M., Wang, L., Frye, M.A., 2019. Pharmacokinetic-Pharmacodynamic interaction associated with venlafaxine-XR remission in patients with major depressive disorder with history of citalopram / escitalopram treatment failure. *J. Affect. Disord.* 246, 62–68. <https://doi.org/10.1016/j.jad.2018.12.021>.
- Areberg, J., Petersen, K.B., Chen, G., Naik, H., 2014. Population pharmacokinetic meta-analysis of vortioxetine in healthy individuals. *Basic Clin. Pharmacol. Toxicol.* 115 (6), 552–559. <https://doi.org/10.1111/bcpt.12256>.

- Blake, C.M., Kharasch, E.D., Schwab, M., Nagele, P., 2013. A meta-analysis of CYP2D6 metabolizer phenotype and metoprolol pharmacokinetics. *Clin. Pharmacol. Ther.* 94 (3), 394–399. <https://doi.org/10.1038/clpt.2013.96>.
- Bell, S.M., et al., 2018. In vitro to in vivo extrapolation for high throughput prioritization and decision making. *Toxicol. In Vitro* 47, 213–227.
- Bhat, V.S., et al., 2017. Evolution of chemical-specific adjustment factors (CSAF) based on recent international experience; increasing utility and facilitating regulatory acceptance. *Crit. Rev. Toxicol.* 47 (9), 729–749.
- Blaauboer, B.J., et al., 2012. The use of biomarkers of toxicity for integrating in vitro hazard estimates into risk assessment for humans. *Altex* 29 (4), 411–425.
- Bromek, E., Haduch, A., Daniel, W.A., 2010. The ability of cytochrome P450 2D isoforms to synthesize dopamine in the brain: An in vitro study. *Eur. J. Pharmacol.* 626 (2–3), 171–178.
- Calabrese, E.J., 1985. Adjustment factors and interindividual variation. *Regul. Toxicol. Pharm.* 5, 190–196.
- Carrão, D.B., et al., 2019. In vitro inhibition of human CYP2D6 by the chiral pesticide fipronil and its metabolite fipronil sulfone: Prediction of pesticide-drug interactions. *Toxicol. Lett.* 313, 196–204.
- Chen, L., Wolf, A.B., Fu, W., Li, L., Akey, J.M., 2020. Identifying and Interpreting Apparent Neanderthal Ancestry in African Individuals. *Cell* 180 (4), 677–687.e16.
- Chiba, K., Kato, M., Ito, T., Suwa, T., Sugiyama, Y., 2012. Inter-individual variability of in vivo CYP2D6 activity in different genotypes. *Drug Metab. Pharmacokinet.* 27 (4), 405–413.
- Cronin-Fenton, D.P., Damkier, P., Lash, T.L., 2014. Metabolism and transport of tamoxifen in relation to its effectiveness: new perspectives on an ongoing controversy. *Future Oncol.* 10 (1), 107–122. <https://doi.org/10.2217/fon.13.168>.
- Cui, Y., Yan, H., Su, Y., Wang, L., Lu, T., Zhang, D., Yue, W., 2020. CYP2D6 Genotype-Based Dose Recommendations for Risperidone in Asian People. *Front. Pharmacol.* 11, 936. <https://doi.org/10.3389/fphar.2020.00936>.
- Cui, Y.M., et al., 2007. Atomoxetine pharmacokinetics in healthy Chinese subjects and effect of the CYP2D6*10 allele. *Br. J. Clin. Pharmacol.* 64 (4), 445–449.
- Curry, S.C., Watts, D.J., Katz, K.D., Bikin, D., Bukaveckas, B.L., 2007. The effect of single-dose tramadol on oxycodone clearance. *J. Emerg. Med.* 33 (4), 407–411.
- Darney, K., 2019. Pharmacokinetic data for CYP3A4 probe substrates in healthy Humans. Zenodo.
- Darney, K., et al., 2020. Bayesian meta-analysis of inter-phenotypic differences in human serum paraoxonase-1 activity for chemical risk assessment. *Environ. Int.* 138, 105609.
- Darney, K., et al., 2019. Inter-ethnic differences in CYP3A4 metabolism: A Bayesian meta-analysis for the refinement of uncertainty factors in chemical risk assessment. *Comput. Toxicol.* 12.
- Dinger, J., Meyer, M.R., Maurer, H.H., 2016. In vitro cytochrome P450 inhibition potential of methylenedioxy-derived designer drugs studied with a two-cocktail approach. *Arch. Toxicol.* 90 (2), 305–318.
- Dorne, J.L.C.M., 2010. Metabolism, variability and risk assessment. *Toxicology* 268 (3), 156–164.

- Dorne, J.L.C.M., Walton, K., Renwick, A.G., 2001a. Human variability in glucuronidation in relation to uncertainty factors for risk assessment. *Food Chem. Toxicol.* 39 (12), 1153–1173.
- Dorne, J.L.C.M., Walton, K., Renwick, A.G., 2001b. Uncertainty factors for chemical risk assessment: Human variability in the pharmacokinetics of CYP1A2 probe substrates. *Food Chem. Toxicol.* 39 (7), 681–696.
- Dorne, J.L.C.M., Walton, K., Renwick, A.G., 2003. Human variability in CYP3A4 metabolism and CYP3A4-related uncertainty factors for risk assessment. *Food Chem. Toxicol.* 41 (2), 201–224.
- Dorne, J.L.C.M., Walton, K., Renwick, A.G., 2004. Human variability for metabolic pathways with limited data (CYP2A6, CYP2C9, CYP2E1, ADH, esterases, glycine and sulphate conjugation). *Food Chem. Toxicol.* 42 (3), 397–421.
- Dorne, J.L.C.M., Walton, K., Renwick, A.G., 2005. Human variability in xenobiotic metabolism and pathway-related uncertainty factors for chemical risk assessment: A review. *Food Chem. Toxicol.* 43 (2), 203–216.
- Dorne, J.L.C.M., Walton, K., Slob, W., Renwick, A.G., 2002. Human variability in polymorphic CYP2D6 metabolism: Is the kinetic default uncertainty factor adequate? *Food Chem. Toxicol.* 40 (11), 1633–1656.
- EFSA Scientific Committee et al., 2017a. Guidance on the use of the weight of evidence approach in scientific assessments. *EFSA Journal*, 15(8).
- EFSA Scientific Committee et al., 2017b. Update: use of the benchmark dose approach in risk assessment. *EFSA Journal*, 15(1).
- Gaedigk, A., Sangkuhl, K., Whirl-Carrillo, M., Klein, T., Leeder, J.S., 2017. Prediction of CYP2D6 phenotype from genotype across world populations. *Genet. Med.* 19 (1), 69–76.
- Gampfer, T.M., et al., 2020. Toxicokinetics and toxicodynamics of the fentanyl homologs cyclopropanoyl-1-benzyl-4-fluoro-4-anilinoipiperidine and furanoyl-1-benzyl-4-anilinoipiperidine. *Arch. Toxicol.* 94 (6), 2009–2025.
- Gan, S.H., Ismail, R., Wan Adnan, W.A., Zulmi, W., 2007. Impact of CYP2D6 genetic polymorphism on tramadol pharmacokinetics and pharmacodynamics. *Mol Diagn Ther* 11 (3), 171–181.
- Garcia-Quetglas, E., et al., 2007. Pharmacokinetics of tramadol enantiomers and their respective phase I metabolites in relation to CYP2D6 phenotype. *Pharmacol. Res.* 55 (2), 122–130.
- Gassó, P., et al., 2013. Relationship between CYP2D6 genotype and haloperidol pharmacokinetics and extrapyramidal symptoms in healthy volunteers. *Pharmacogenomics* 14 (13), 1551–1563.
- Ginsberg, G., et al., 2002. Evaluation of child/adult pharmacokinetic differences from a database derived from the therapeutic drug literature. *Toxicol. Sci.* 66 (2), 185–200.
- Haduch, A., Daniel, W.A., 2018. The engagement of brain cytochrome P450 in the metabolism of endogenous neuroactive substrates: a possible role in mental disorders. *Drug Metab. Rev.* 50 (4), 415–429.
- He, Z.X., Chen, X.W., Zhou, Z.W., Zhou, S.F., 2015. Impact of physiological, pathological and environmental factors on the expression and activity of human cytochrome P450 2D6 and implications in precision medicine. *Drug Metab. Rev.* 47 (4), 470–519.
- Hiroi, T., et al., 2001. Progesterone oxidation by cytochrome P450 2D isoforms in the brain. *Endocrinology* 142 (9), 3901–3908.
- Ingelman-Sundberg, M., Persson, A., Jukic, M.M., 2014. Polymorphic expression of CYP2C19 and CYP2D6 in the developing and adult human brain causing variability in cognition, risk for depression and suicide: the search for the endogenous substrates. *Pharmacogenomics* 15 (15), 1841–1844.
- Ito, K., Hallifax, D., Obach, R.S., Houston, J.B., 2005. Impact of parallel pathways of drug elimination and multiple cytochrome P450 involvement on drug-drug interactions: CYP2D6 paradigm. *Drug Metab. Dispos.* 33 (6), 837.
- Johansson, I., Ingelman-Sundberg, M., 2011. Genetic polymorphism and toxicology—with emphasis on cytochrome P450. *Toxicol. Sci.* 120 (1), 1–13.
- Kasteel, E.E.J., Darney, K., Kramer, N.I., Dorne, J., Lautz, L.S., 2020. Human variability in isoform-specific UDP-glucuronosyltransferases: markers of acute and chronic exposure, polymorphisms and uncertainty factors. *Arch. Toxicol.* 94 (8), 2637–2661.
- Kato, D., et al., 2005. CYP2D6 gene deletion allele in patients with neuroleptic malignant syndrome: preliminary report. *Psychiatry Clin. Neurosci.* 59 (4), 504–507.
- Kirchheiner, J., Keulen, J.T., Bauer, S., Roots, I., Brockmoller, J., 2008. Effects of the CYP2D6 gene duplication on the pharmacokinetics and pharmacodynamics of tramadol. *J. Clin. Psychopharmacol.* 28 (1), 78–83.
- Kuhn, U.D., et al., 2007. Reboxetine and cytochrome P450 - Comparison with paroxetine treatment in humans. *Int. J. Clin. Pharmacol. Ther.* 45 (1), 36–46.
- Li, S., Lin, H., Sun, W., Wang, Y., Ding, Y., Zhao, H., Liu, S., 2017. A meta-analysis of the effect of CYP2D6 polymorphism on the pharmacokinetics and pharmacodynamics of metoprolol. *Int. J. Clin. Pharmacol. Ther.* 55 (6), 483–492. <https://doi.org/10.5414/cp202545>.
- Lin, X.Q., Wang, P., Cai, W.K., Xu, G.L., Yang, M., Zhou, M.D., Sun, M., He, F., He, G.H., 2019. The Associations Between CYP2D6 Metabolizer Status and Pharmacokinetics and Clinical Outcomes of Venlafaxine: A Systematic Review and Meta-Analysis. *Pharmacopsychiatry* 52 (5), 222–231. <https://doi.org/10.1055/a-0792-1340>.
- Linares, O.A., Fudin, J., Daly, A.L., Boston, R.C., 2015. Individualized Hydrocodone Therapy Based on Phenotype, Pharmacogenetics, and Pharmacokinetic Dosing. *Clin. J. Pain* 31 (12), 1026–1035. <https://doi.org/10.1097/ajp.0000000000000214>.
- Lokke, H., 2010. Novel methods for integrated risk assessment of cumulative stressors — Results from the NoMiracle project. *Sci. Total Environ.* 408 (18), 3719–3724.
- Malhotra, B., Guan, Z., Wood, N., Gandelman, K., 2008. Pharmacokinetic profile of fesoterodine. *Int. J. Clin. Pharmacol. Ther.* 46 (11), 556–563.
- Malhotra, B.K., Wood, N., Sachse, R., 2009. Influence of age, gender, and race on pharmacokinetics, pharmacodynamics, and safety of fesoterodine. *Int. J. Clin. Pharmacol. Ther.* 47 (9), 570–578.
- Magliocco, G., Thomas, A., Jules Desmeules, J., Daali, Y., 2019. Phenotyping of Human CYP450 Enzymes by Endobiotics: Current Knowledge and Methodological Approaches. *Clin. Pharmacokinet.* 58 (11):1373–1391.
- Mann, A., et al., 2012. The neuroprotective enzyme CYP2D6 increases in the brain with age and is lower in Parkinson's disease patients. *Neurobiol. Aging* 33 (9), 2160–2171.
- Maruf, Abdullah Al, Stein, Kiera, Arnold, Paul D., Aitchison, Katherine J., Müller, Daniel J., Bousman, Chad, 2020. CYP2D6 and Antipsychotic Treatment Outcomes in Children and Youth: A Systematic Review. *J. Child Adolesc. Psychopharmacol.* 31 (1), 33–45. <https://doi.org/10.1089/cap.2020.0093>.
- Meloche, M., Khazaka, M., Kassem, I., Barhdadi, A., Dubé, M.P., de Denu, S., 2020. CYP2D6 polymorphism and its impact on the clinical response to metoprolol: A systematic review and meta-analysis. *Br. J. Clin. Pharmacol.* 86 (6), 1015–1033. <https://doi.org/10.1111/bcp.14247>.
- Miksys, S., Tyndale, R.F., 2006. Nicotine induces brain CYP enzymes: relevance to Parkinson's disease. In: Riederer, P., Reichmann, H., Youdim, M.B.H., Gerlach, M. (Eds.), *Parkinson's Disease and Related Disorders*. Springer Vienna, Vienna, pp. 177–180.
- Milosavljevic, F., Bukvic, N., Pavlovic, Z., Miljevic, C., Pešić, V., Molden, E., Ingelman-Sundberg, M., Leucht, S., Jukic, M.M., 2021. Association of CYP2C19 and CYP2D6 Poor and Intermediate Metabolizer Status With Antidepressant and Antipsychotic Exposure: A Systematic Review and Meta-analysis. *JAMA Psychiatry* 78 (3), 270–280. <https://doi.org/10.1001/jamapsychiatry.2020.3643>.
- Miller, R.T., Miksys, S., Hoffmann, E., Tyndale, R.F., 2014. Ethanol self-administration and nicotine treatment increase brain levels of CYP2D in African green monkeys. *Br. J. Pharmacol.* 171 (12), 3077–3088.
- Naumann, B.D., et al., 1997. Use of toxicokinetic and toxicodynamic data to reduce uncertainties when setting occupational exposure limits for pharmaceuticals. *Human Ecol. Risk Assess.* Int. J. 3 (4), 555–565.
- Nichols, A.I., et al., 2011. The pharmacokinetics and safety of desvenlafaxine in subjects with chronic renal impairment. *Int. J. Clin. Pharmacol. Ther.* 49 (1), 3–13.
- Niwa, T., et al., 2008. Effect of psychotropic drugs on the 21-hydroxylation of neurosteroids, progesterone and allopregnanolone, catalyzed by rat CYP2D4 and human CYP2D6 in the brain. *Biol. Pharm. Bull.* 31 (3), 348–351.
- Pedersen, A.J., Petersen, T.H., Linnet, K., 2013. In Vitro Metabolism and Pharmacokinetic Studies on Methylone. *Drug Metab. Dispos.* 41 (6), 1247.
- Pedersen, R.S., Damkier, P., Brosen, K., 2006. Enantioselective pharmacokinetics of tramadol in CYP2D6 extensive and poor metabolizers. *Eur. J. Clin. Pharmacol.* 62 (7), 513–521.
- Plummer, M., 2003. JAGS: a program for analysis of Bayesian graphical models using Gibbs sampling. In: Hornik, K., Leisch, F., Zeileis, A. (Eds.), *Proceedings of the 3rd International Workshop on Distributed Statistical Computing*, Vienna, Austria, pp. 1–10.
- Province, M.A., Goetz, M.P., Brauch, H., Flockhart, D.A., Hebert, J.M., Whaley, R., Suman, V.J., Schroth, W., Winter, S., Zembutsu, H., Mushiroya, T., Newman, W.G., Lee, M.T., Ambrosone, C.B., Beckmann, M.W., Choi, J.Y., Dieudonné, A.S., Fasching, P.A., Ferraldeschi, R., Gong, L., Haschke-Becher, E., Howell, A., Jordan, L. B., Hamann, U., Kiyotani, K., Krippel, P., Lambrechts, D., Latif, A., Langenlehner, U., Lorizio, W., Neven, P., Nguyen, A.T., Park, B.W., Purdie, C.A., Quinlan, P., Renner, W., Schmidt, M., Schwab, M., Shin, J.G., Stingl, J.C., Wegman, P., Wingren, S., Wu, A.H., Ziv, E., Zirpoli, G., Thompson, A.M., Jordan, V.C., Nakamura, Y., Altman, R.B., Ames, M.M., Weinsilboum, R.M., Eichelbaum, M., Ingle, J.N., Klein, T.E., 2014. CYP2D6 genotype and adjuvant tamoxifen: meta-analysis of heterogeneous study populations. *Clin. Pharmacol. Ther.* 95 (2), 216–227. <https://doi.org/10.1038/clpt.2013.186>.
- Renwick, A.G., Lazarus, N.R., 1998. Human variability and noncancer risk assessment - An analysis of the default uncertainty factor. *Regul. Toxicol. Pharm.* 27 (1), 3–20.
- Samer, C.F., et al., 2010. Genetic polymorphisms and drug interactions modulating CYP2D6 and CYP3A activities have a major effect on oxycodone analgesic efficacy and safety. *Br. J. Pharmacol.* 160 (4), 919–930.
- Sharma, A., et al., 2010. Toward optimal treatment in women: The effect of sex on metoprolol-diphenhydramine interaction. *J. Clin. Pharmacol.* 50 (2), 214–225.
- Singh, M., Khanna, V.K., Shukla, R., Parmar, D., 2010. Association of polymorphism in cytochrome P450 2D6 and N-acetyltransferase-2 with Parkinson's disease. *Dis. Markers* 28 (2), 87–93.
- Slanař, O., et al., 2007. Miotic action of tramadol is determined by CYP2D6 genotype. *Physiol. Res.* 56 (1), 129–136.
- Steffens, M., Hübner, T., Scholl, C., Viviani, R., Stingl, J.C., 2019. Was the Neanderthal a poor metabolizer of CYP2D6. In *EMBO-EMBL Symposium: Reconstructing the Human Past Using Ancient and Modern Genomics*.
- Steuer, A.E., Williner, E., Staeheli, S.N., Kraemer, T., 2017. Studies on the metabolism of the fentanyl-derived designer drug butyrfentanyl in human in vitro liver preparations and authentic human samples using liquid chromatography-high resolution mass spectrometry (LC-HRMS). *Drug Test. Anal.* 9 (7), 1085–1092.
- Stingl, J., Viviani, R., 2015. Polymorphism in CYP2D6 and CYP2C19, members of the cytochrome P450 mixed-function oxidase system, in the metabolism of psychotropic drugs. *J. Intern. Med.* 277 (2), 167–177. <https://doi.org/10.1111/joim.12317>.
- Stodtmann, S., Nuthalapati, S., Eckert, D., Kasichayana, S., Joshi, R., Bach, B.A., Mensing, S., Menon, R., Xiong, H., 2021. A Population Pharmacokinetic Meta-analysis of Veliparib, a PARP Inhibitor, Across Phase I/II/III Trials in Cancer Patients. *J. Clin. Pharmacol.* <https://doi.org/10.1002/jcph.1875>.
- Tang, J., et al., 2001. Metabolism of chlorpyrifos by human cytochrome P450 isoforms and human, mouse, and rat liver microsomes. *Drug Metab. Dispos.* 29 (9), 1201–1204.

- Taylor, C., et al., 2020. A Review of the Important Role of CYP2D6 in Pharmacogenomics. *Genes (Basel)* 11 (11).
- Tay-Sontheimer, J., Shireman, L.M., Beyer, R.P., Senn, T., Witten, D., Pearce, R.E., Gaedigk, A., Gana Fomban, C.L., Lutz, J.D., Isoherranen, N., Thummel, K.E., Fiehn, O., Leeder, J.S., Lin, Y.S., 2014. Detection of an endogenous urinary biomarker associated with CYP2D6 activity using global metabolomics. *Pharmacogenomics* 2014 Dec;15(16):1947-62. doi: 10.2217/pgs.14.155.
- Tracy, T.S., Chaudhry, A.S., Prasad, B., Thummel, K.E., Schuetz, E.G., Zhong, X.-B., 2016. Interindividual variability in cytochrome P450-mediated drug metabolism. *Drug Metab Dispos Biol Fate Chem* 44, 343–351.
- Truhaut, R., 1991. The concept of the acceptable daily intake: An historical review. *Food Addit. Contam.* 8 (2), 151–162.
- Villanea, F.A., Schraiber, J.G., 2019. Multiple episodes of interbreeding between Neanderthal and modern humans. *Nat. Ecol. Evol.* 3 (1), 39–44.
- Viviani, R., et al., 2020. Effects of genetic variability of CYP2D6 on neural substrates of sustained attention during on-task activity. *Transl. Psychiatry* 10 (1), 338.
- Voronov, P., Przybylo, H.J., Jagannathan, N., 2007. Apnea in a child after oral codeine: a genetic variant - an ultra-rapid metabolizer. *Paediatr Anaesth.* 17 (7), 684–687.
- Wagmann, L., et al., 2019. Phenethylamine-derived new psychoactive substances 2C-E-FLY, 2C-EF-FLY, and 2C-T-7-FLY: Investigations on their metabolic fate including isoenzyme activities and their toxicological detectability in urine screenings. *Drug Test. Anal.* 11 (10), 1507–1521.
- Walpole, S.C., et al., 2012. The weight of nations: An estimation of adult human biomass. *BMC Public Health* 12 (1).
- Wen, Q.H., Zhang, Z., Cai, W.K., Lin, X.Q., He, G.H., 2020. The Associations Between CYP2D6*10 C188T Polymorphism and Pharmacokinetics and Clinical Outcomes of Tramadol: A Systematic Review and Meta-analysis. *Pain Med.* 21 (12), 3679–3690. <https://doi.org/10.1093/pm/pnaa140>.
- Wiecek, W., Dorne, J.-L., Quignot, N., Bechaux, C., Amzal, B., 2019. A generic Bayesian hierarchical model for the meta-analysis of human population variability in kinetics and its applications in chemical risk assessment. *Comput. Toxicol.* 12, 100106.
- Wilde, M., et al., 2019. Metabolic Pathways and Potencies of New Fentanyl Analogs. *Front. Pharmacol.* 10 (238).
- Woo, S.I., Hansen, L.A., Yu, X., Mallory, M., Masliah, E., 1999. Alternative splicing patterns of CYP2D genes in human brain and neurodegenerative disorders. *Neurology* 53 (7), 1570–1572.
- Wu, S.B., et al., 2015. Impact of CYP2D6 Polymorphisms on Postoperative Fentanyl Analgesia in Gastric Cancer Patients. *Genet. Test. Mol. Biomarkers* 19 (5), 248–252.
- Yang, Y., Botton, M.R., Scott, E.R., Scott, S.A., 2017. Sequencing the CYP2D6 gene: from variant allele discovery to clinical pharmacogenetic testing. *Pharmacogenomics* 18 (7), 673–685.
- Yang, M., et al., 2019. Intestinal and hepatic biotransformation of pyrrolizidine alkaloid N-oxides to toxic pyrrolizidine alkaloids. *Arch. Toxicol.* 93 (8), 2197–2209.
- Yoo, H.D., Cho, H.Y., Lee, S.N., Yoon, H., Lee, Y.B., 2012. Population pharmacokinetic analysis of risperidone and 9-hydroxyrisperidone with genetic polymorphisms of CYP2D6 and ABCB1. *J. Pharmacokinet Pharmacodyn.* 39 (4), 329–341.
- Yu, A.M., et al., 2003. Regeneration of serotonin from 5-methoxytryptamine by polymorphic human CYP2D6. *Pharmacogenetics* 13 (3), 173–181.
- Zhang, L., Brown, S.J., Shan, Y., Lee, A.M., Allen, J.D., Eum, S., de Leon, J., Bishop, J.R., 2020. CYP2D6 Genetic Polymorphisms and Risperidone Pharmacokinetics: A Systematic Review and Meta-analysis. *Pharmacotherapy* 40 (7), 632–647. <https://doi.org/10.1002/phar.2434>.
- Zhang, X., Xiang, Q., Zhao, X., Ma, L., Cui, Y., 2019. Association between aripiprazole pharmacokinetics and CYP2D6 phenotypes: A systematic review and meta-analysis. *J. Clin. Pharm. Ther.* 44 (2), 163–173. <https://doi.org/10.1111/jcpt.12780>.
- Zhou, S.-F., 2009. Polymorphism of Human Cytochrome P450 2D6 and Its Clinical Significance. *Clin. Pharmacokinet.* 48 (11), 689–723.
- Zivković, M., Mihaljević-Peles, A., Sagud, M., Silić, A., Mihanović, M., 2010. The role of CYP2D6 and TaqI A polymorphisms in malignant neuroleptic syndrome: two case reports with three episodes. *Psychiatr Danub* 22 (1), 112–116.