CYP2D6 function moderates the pharmacokinetics and pharmacodynamics of 3,4-methylene-dioxymethamphetamine in a controlled study in healthy individuals

Yasmin Schmid[a], Patrick Vizeli[a], Cédric M. Hysek[a], Katharina Prestin[b], Henriette E. Meyer zu Schwabedissen[b] and Matthias E. Liechti[a]

The role of genetic polymorphisms in cytochrome (CYP) 2D6 involved in the metabolism of 3,4-methylene-dioxymethamphetamine (MDMA, ecstasy) is unclear. Effects of genetic variants in CYP2D6 on the pharmacokinetics and pharmacodynamic effects of MDMA were characterized in 139 healthy individuals (70 men, 69 women) in a pooled analysis of eight double-blind, placebo-controlled crossover studies. In CYP2D6 poor metabolizers, the maximum concentrations (C_{max}) of MDMA and its active metabolite 3,4-methylene-dioxyamphetamine were + 15 and + 50% higher, respectively, compared with extensive metabolizers and the C_{max} of the inactive metabolite 4-hydroxy-3-methoxymethamphetamine was 50–70% lower. Blood pressure and subjective drug effects increased more rapidly after MDMA administration in poor metabolizers than in extensive metabolizers. In conclusion, the disposition of MDMA and its effects in humans are altered by polymorphic CYP2D6 activity, but the effects are small because of the autoinhibition of CYP2D6.

Pharmacogenetics and Genomics 2016, **26**:397–401

Keywords: cytochrome P450, CYP2D6, 4-hydroxy-3-methoxymethamphetamine, 3,4-methylene-dioxyamphetamine, 3,4-methylene-dioxymethamphetamine, pharmacokinetics

[a]Division of Clinical Pharmacology and Toxicology, Departments of Biomedicine and Clinical Research, University Hospital Basel and [b]Department of Pharmaceutical Sciences, University of Basel, Basel, Switzerland

Correspondence to Matthias E. Liechti, MD, MAS, Division of Clinical Pharmacology and Toxicology, University Hospital Basel, Hebelstrasse 2, Basel CH-4031, Switzerland
Tel: + 41 613 286 868; fax: + 41 612 654 560; e-mail: matthias.liechti@usb.ch

Received 4 February 2016 **Accepted** 5 May 2016

Interindividual differences in the clinical toxicity of 3,4-methylene-dioxymethamphetamine (MDMA) may result from alterations in drug-metabolizing enzymes, such as cytochrome P450 monooxygenases (CYPs), that are involved in the metabolism of MDMA [1,2] shown in Supplementary Fig. S1, Supplemental digital content 1, *http://links.lww.com/FPC/B41*.

Only limited controlled data are available on the pharmacogenetics/toxicogenetics of MDMA [2]. The aim of the present study was to investigate the role of CYP2D6 in the PK of MDMA in a prospectively designed pooled analysis of eight double-blind, placebo-controlled, crossover studies in a total of 139 healthy individuals (methods shown in Supplemental digital content 1, *http://links.lww.com/FPC/B41*). This is the first study with a meaningful sample size and the inclusion of several individuals with relevantly impaired function. We also performed both genotyping and phenotyping and used genetic activity scores [3] for CYP2D6 activity classification.

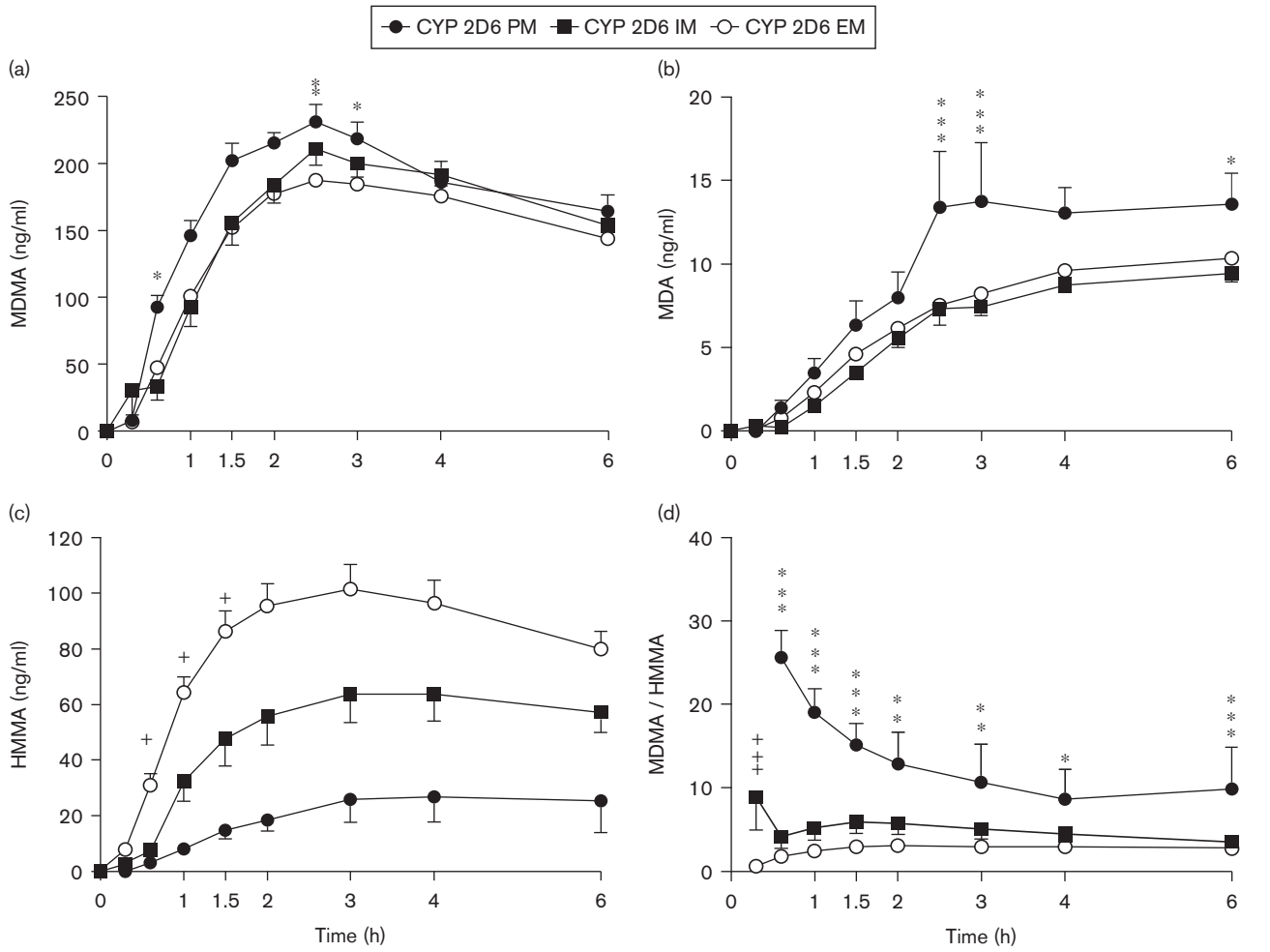
We found that CYP2D6 activity significantly altered plasma MDMA levels up to 3 h after drug administration (i.e. during drug absorption/distribution), but not beyond 3 h (i.e. during drug elimination; Supplementary Table S1, Supplemental digital content 1, *http://links.lww.com/FPC/B41*, Fig. 1a and Supplementary Fig. S2c, Supplemental digital content 1, *http://links.lww.com/FPC/B41*). A significant main effect of the CYP2D6 genotype on the C_{max} of MDMA was found ($F_{2,136} = 4.19$, $P = 0.02$), with higher C_{max} values in CYP2D6 poor metabolizers (PMs) compared with extensive metabolizers (EMs; $P = 0.049$; Supplementary Fig. S1a, Supplemental digital content 1, *http://links.lww.com/FPC/B41*). The CYP2D6 activity score similarly altered the C_{max} of MDMA (Supplementary Table S1 and Supplementary Fig. S2b, Supplemental digital content 1, *http://links.lww.com/FPC/B41*). MDMA area under the concentration–time curve up to 6 h (AUC_6) values also varied across CYP2D6 genotype groups ($F_{2,136} = 5.25$, $P < 0.01$), with PMs showing higher AUC_6 values compared with EMs ($P < 0.01$; Supplementary Fig. S2d, Supplemental digital content 1, *http://links.lww.com/FPC/B41*). MDMA AUC_6 values also differed between genotype-based CYP2D6 activity groups (Supplementary Table S1 and Supplementary Fig. S2e, Supplemental digital content 1, *http://links.lww.com/FPC/B41*). The CYP 2D6 genotype

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's website (*www.pharmacogeneticsandgenomics.com*).

1744-6872 Copyright © 2016 Wolters Kluwer Health, Inc. All rights reserved.

DOI: 10.1097/FPC.0000000000000231

Fig. 1



CYP2D6 phenotypes predicted by genotyping altered the pharmacokinetics of MDMA (a), MDA (b), HMMA (c), and the MDMA/HMMA ratio (d). Lower CYP2D6 function as in PMs resulted in higher MDMA (a) and MDA (b) plasma levels, lower HMMA plasma levels (c), and higher MDMA/HMMA plasma concentration ratios (d) compared with higher CYP2D6 function as in EMs. The data are expressed as the mean ± SEM in seven PMs, 19 IMs, and 113 EMs for MDMA and MDA. MDMA was administered at $t = 0$ h. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ for PMs compared with EMs and $^{+}P < 0.05$ for EMs compared with IMs at the corresponding time. CYP2D6, cytochrome 2D6; EMs, extensive metabolizers; HMMA, 4-hydroxy-3-methoxymethamphetamine; IM, intermediate metabolizers; MDA, 3,4-methylene-dioxyamphetamine; MDMA, 3,4-methylene-dioxymethamphetamine; PMs, poor metabolizers.

altered the concentration–time curve of the minor active metabolite 3,4-methylene-dioxyamphetamine (MDA) (Fig. 1b). The C_{max} and AUC_6 of MDA varied across genotypes ($F_{2,136} = 8.82$, $P < 0.01$, and $F_{2,136} = 9.09$, $P < 0.001$, respectively), which were higher in PMs compared with intermediate metabolizers (IMs; $P < 0.001$) and EMs ($P < 0.001$; Supplementary Fig. S3a and d, Supplemental Digital Content 1, *http://links.lww.com/FPC/B41*). The C_{max} and AUC_6 of MDA were also higher in individuals with a CYP2D6 activity score of 0 compared with all of the other CYP2D6 activity groups (Supplementary Table S1 and Supplementary Fig. S2b, c, e, Supplemental digital content 1, *http://links.lww.com/FPC/B41*). The CYP2D6 genotype influenced the concentration–time curve of the inactive metabolite 4-hydroxy-3-methoxymethamphetamine (HMMA; Fig. 1c). The CYP2D6 genotype altered the C_{max} and AUC_6 of HMMA ($F_{2,73} = 3.50$, $P = 0.03$ and 5.22, $P < 0.01$; Supplementary Table S1 and Supplementary Fig. S4a and d, Supplemental digital content 1, *http://links.lww.com/FPC/B41*). C_{max} and AUC_6 of HMMA were higher in individuals with high CYP2D6 activity (activity score of 2) compared with individuals with low activity (activity score of 0.5; Supplementary Table S1 and Supplementary Fig. S4b, c, e, Supplemental digital content 1, *http://links.lww.com/FPC/B41*). The CYP2D6 genotype altered the unconjugated HMMA concentrations similar to those of HMMA (Supplementary Table S2 and Supplementary Fig. S5, Supplemental digital content 1, *http://links.lww.com/FPC/B41*). The CYP2D6

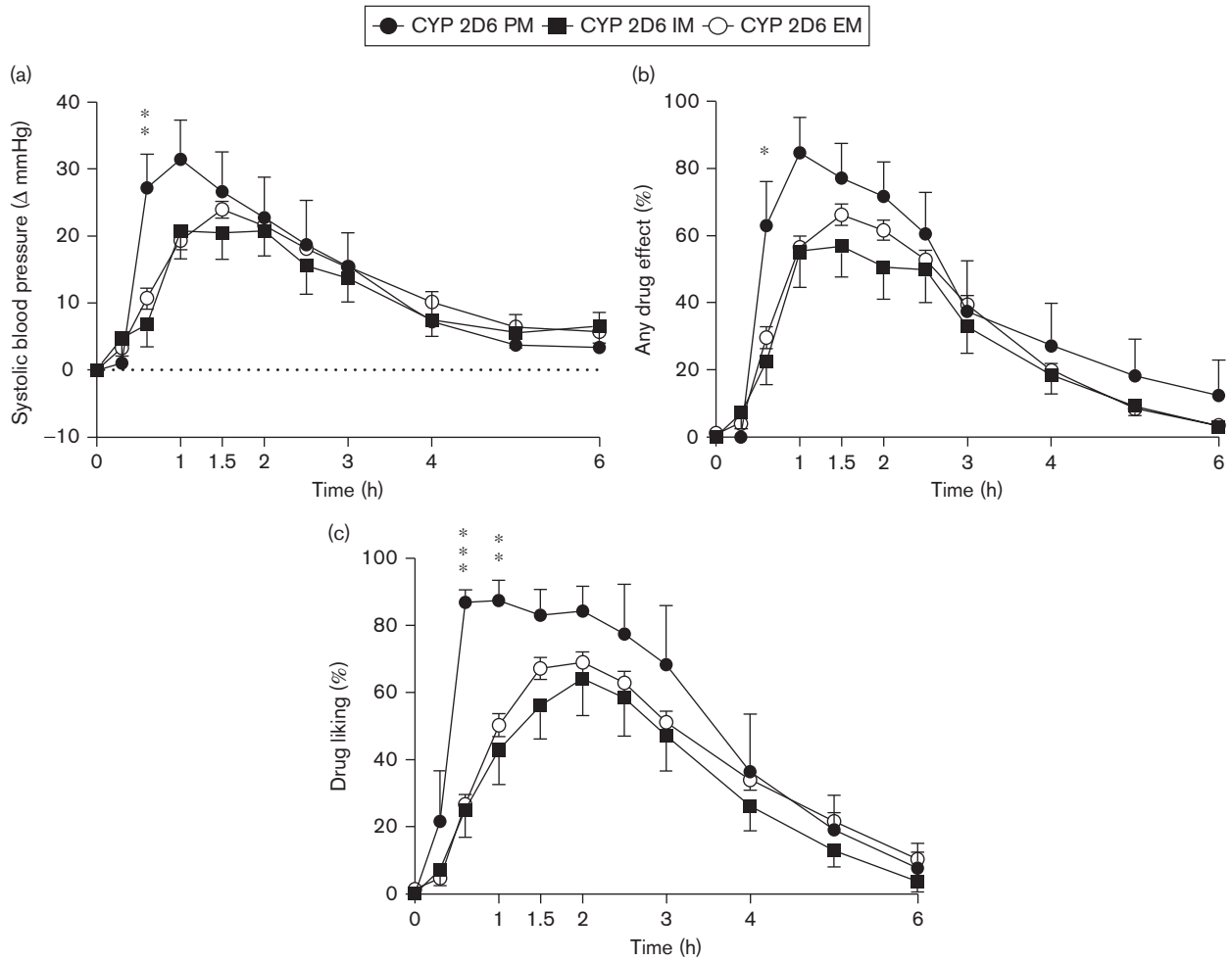
genotype altered the MDMA/HMMA C_{max} and AUC_6 ratios ($F_{2,73} = 10.98$ and 10.08, both $P < 0.001$; Fig. 1d). CYP2D6 PMs had higher MDMA/HMMA C_{max} and AUC_6 ratios compared with IMs ($P < 0.01$ and $P < 0.05$) and EMs (both $P < 0.001$; Fig. 1d, and Supplementary Fig. S6a and d, Supplemental digital content 1, *http://links.lww.com/FPC/B41*). Individuals with a CYP2D6 activity score of 2 had lower MDMA/HMMA C_{max} and AUC_6 ratios compared with individuals with activity scores of 0, 0.5, and 1 (Supplementary Table S1 and Supplementary Fig. S6b, c, e, Supplemental digital content 1, *http://links.lww.com/FPC/B41*). The effects of CYP2D6 on the biotransformation of MDMA to HMMA over time are also evident in the hysteresis plots in Supplementary Fig. S8, Supplemental digital content 1, *http://links.lww.com/FPC/B41*. A correlation was found between the AUC_6 values of MDMA and MDA ($R_s = 0.18$, $P < 0.05$, $N = 139$), indicating that higher MDMA levels resulted in higher MDA levels. In contrast, higher AUC_6 values of MDMA were negatively correlated with those of HMMA ($R_s = -0.33$, $P < 0.01$, $N = 76$) or unconjugated HMMA ($R_s = -0.39$, $P < 0.001$, $N = 124$), consistent with the impaired conversion of MDMA into HMMA.

CYP2D6 activity influenced both the cardiovascular and the psychotropic responses to MDMA. Both the MDMA-induced blood pressure response and subjective drug effects increased more rapidly in genotype-based CYP2D6 PMs compared with IMs and EMs (Fig. 2), reflected by group differences early in time after MDMA administration, whereas maximal effects did not differ. Elevations in systolic blood pressure were greater in PMs compared with IMs ($P = 0.02$) and EMs ($P = 0.01$) at 0.6 h ($F_{2,135} = 3.50$, $P = 0.03$) and also tended to be greater at 1 h ($F_{2,135} = 2.49$, $P = 0.09$) after drug administration. Subjective 'any drug effect' ratings were higher in PMs compared with both IMs and EMs at 0.6 h ($P < 0.05$). 'Drug liking' ratings were higher in PMs compared with both IMs and EMs at 0.6 h ($P < 0.001$) and 1 h ($P < 0.01$) and tended to be higher at 1.5 h. No effects of the CYP2D6 genotype were found on heart rate or body temperature. The MDMA/HMMA ratio at 0.6 h, which inversely reflects CYP2D6 activity, was associated with the MDMA-induced elevations in systolic blood pressure ($R_s = 0.41$, $P < 0.001$, $N = 76$), any drug effect ($R_s = 0.42$, $P < 0.001$), and drug liking ($R_s = 0.40$, $P < 0.001$) at 0.6 h. The association remained significant for systolic blood pressure up to 1.5 h and for any drug effect and drug liking up to 4 and 6 h, respectively.

As expected, the CYP2D6 phenotype was associated with the CYP2D6 genotype. The DM/DX ratio in the group of PMs with an activity score 0 was significantly greater than in all of the other activity score groups, but these ratios did not differ between IMs and the different EM groups or within the EM groups (Supplementary Table S1 and Supplementary Fig. S9, Supplemental digital content 1, *http://links.lww.com/FPC/B41*). Despite some discordance between individual genotypes and phenotypes, the effects of the CYP2D6 genotype (Fig. 1) and the CYP2D6 phenotype (Supplementary Fig. S10, Supplemental digital content 1, *http://links.lww.com/FPC/B41*) groups on MDMA and metabolite plasma–time curves were very similar. Additional findings are presented in the Supplementary material, Supplemental digital content 1, *http://links.lww.com/FPC/B41*.

Taken together, the present study found an increase in MDMA exposure and more rapid increases in the cardiostimulant and psychotropic effects of MDMA in individuals with poor CYP2D6 function. The mean C_{max} levels of MDMA in the seven CYP2D6 PMs in the present study were only 1.15 times higher than in 111 EMs, and differences in the PK or subjective and blood pressure responses to MDMA were only present in the first hour after MDMA administration. Thus, interindividual differences in CYP2D6 function have a small and transient effect on the plasma concentration of MDMA and its pharmacodynamic effects. These findings are consistent with the mechanism-based inhibition of CYP2D6 by MDMA, turning all individuals into functional CYP2D6 PMs within 1 h after MDMA administration [4,5]. Consistent with our experimental data, a physiologically based PK model estimated that the absence of CYP2D6 function in PMs would increase MDMA C_{max} levels by only 36% [4]. Consistent with the findings in genetically impaired CYP2D6 PMs in the present study, the pharmacological inhibition of CYP2D6 function increased the C_{max} and AUC values of MDMA by 15–20 and 10–30%, respectively [6,7]. The mean HMMA AUC_6 in CYP2D6 PMs was four times lower (24%) compared with EMs. This more pronounced effect of the CYP2D6 genotype on HMMA concentrations compared with MDMA concentrations could be clinically relevant because metabolites that are formed by CYP2D6, including HHMA and HMMA, have been implicated in MDMA-induced neurotoxicity and hepatotoxicity [8], hyponatremia, and hyperthermia [9]. Low CYP2D6 activity was associated with higher MDA levels. Although CYP2D6 has been shown to be involved in the *N*-demethylation of MDMA to MDA in-vitro [10], individuals with low CYP2D6 function appear to produce more MDA because the main metabolic pathway of CYP2D6-mediated MDMA-HMMA conversion is less active, thus resulting in higher MDMA levels and compensatory MDA formation. Altogether, statistically significant but only moderate clinical effects of CYP2D6 genetics or pharmacological CYP2D6 inhibition on MDMA disposition were found, effects that are attributable to the self-inhibition of CYP2D6 and phenoconversion. CYP2D6 PMs were not overrepresented in fatalities associated with ecstasy [11]. However, individuals may be at increased risk when more than one CYP system is genetically impaired or pharmacologically

Fig. 2



CYP2D6 phenotypes predicted by genotyping modulated the blood pressure and subjective responses to MDMA. Systolic blood pressure (a) and subjective effects, including any drug effects (b) and drug liking (c), increased more rapidly in CYP2D6 PMs compared with IMs and EMs. The data are expressed as the mean ± SEM in seven PMs, 19 IMs, and 113 EMs. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, PMs compared with IMs or EMs at the corresponding time. CYP2D6, cytochrome 2D6; EMs, extensive metabolizers; IM, intermediate metabolizers; MDMA, 3,4-methylenedioxymethamphetamine; PMs, poor metabolizers.

inhibited. Severe or fatal MDMA toxicity and high plasma exposure to MDMA were noted in cases of co-use with ritonavir, which blocks not only CYP2D6 but also CYP2B6 and CYP3A4. In fact, because CYP2D6 is inhibited by MDMA itself in all individuals, polymorphisms in other CYPs (e.g. CYP1A2, CYP2C19, and CYP2B6) and the pharmacological inhibition of CYP3A4 may actually be more clinically relevant than CYP2D6 genetics. Supporting this view, CYP1A2 function was shown to increase after MDMA administration in another study [12], which possibly compensated for the inhibition of CYP2D6 function by MDMA. We have assessed the effects of CYPA12, CYP2C19, and CYP2B6 polymorphisms on the PK of MDMA in this study and the findings will be published separately. There were no moderating effects of these polymorphisms on the effects of CYP2D6.

The present study has limitations. We analyzed a pooled sample of different studies and different doses and the different CYP2D6 activity groups were not equally represented across studies and dose groups. In addition, there were only seven PMs and all were in the high-dose group. However, an analysis of the high-dose group only produced similar results as the analysis of the total sample.

In conclusion, the present study evaluated the CP2D6 pharmacogenetics of the PK of an important recreational substance in a unique cohort that included a relatively large number of individuals and a wide spectrum of CYP functions, including CYP2D6 PMs. The study found

only moderate increases in the plasma exposure to MDMA and its active metabolite MDA and a more rapid onset of associated blood pressure and psychotropic effects in individuals with poor CYP2D6 function. Although CYP2D6 PMs may be at increased risk for MDMA toxicity, the self-inhibition of CYP2D6 reduces the impact of the CYP2D6 genotype on MDMA PK.

Acknowledgements

This study was supported by the Swiss National Science Foundation (grant no. 320030_1449493).

Conflicts of interest

There are no conflicts of interest.

References

1 De la Torre R, Yubero-Lahoz S, Pardo-Lozano R, Farre M. MDMA, methamphetamine, and CYP2D6 pharmacogenetics: what is clinically relvant? *Front Genet* 2012; **3**:1–6.
2 Rietjens SJ, Hondebrink L, Westerink RH, Meulenbelt J. Pharmacokinetics and pharmacodynamics of 3,4-methylenedioxymethamphetamine (MDMA): interindividual differences due to polymorphisms and drug–drug interactions. *Crit Rev Toxicol* 2012; **42**:854–876.
3 Hicks JK, Swen JJ, Thorn CF, Sangkuhl K, Kharasch ED, Ellingrod VL, *et al*. Clinical pharmacogenetics implementation consortium guideline for CYP2D6 and CYP2C19 genotypes and dosing of tricyclic antidepressants. *Clin Pharmacol Ther* 2013; **93**:402–408.
4 Yang J, Jamei M, Heydari A, Yeo KR, de la Torre R, Farré M, *et al*. Implications of mechanism-based inhibition of CYP2D6 for the pharmacokinetics and toxicity of MDMA. *J Psychopharmacol* 2006; **20**:842–849.
5 O'Mathúna B, Farré M, Rostami-Hodjegan A, Yang J, Cuyàs E, Torrens M, *et al*. The consequences of 3,4-methylenedioxymethamphetamine induced CYP2D6 inhibition in humans. *J Clin Psychopharmacol* 2008; **28**:523–529.
6 Hysek CM, Simmler LD, Ineichen M, Grouzmann E, Hoener MC, Brenneisen R, *et al*. The norepinephrine transporter inhibitor reboxetine reduces stimulant effects of MDMA ('ecstasy') in humans. *Clin Pharmacol Ther* 2011; **90**:246–255.
7 Hysek CM, Simmler LD, Nicola V, Vischer N, Donzelli M, Krähenbühl S, *et al*. Duloxetine inhibits effects of MDMA ('ecstasy') in vitro and in humans in a randomized placebo-controlled laboratory study. *PloS One* 2012; **7**:e36476.
8 Antolino-Lobo I, Meulenbelt J, Nijmeijer SM, Scherpenisse P, van den Berg M, van Duursen MB. Differential roles of phase I and phase II enzymes in 3,4-methylendioxymethamphetamine-induced cytotoxicity. *Drug Metab Dispos* 2010; **38**:1105–1112.
9 De la Torre R, Farré M, Mathúna BO, Roset PN, Pizarro N, Segura M, *et al*. MDMA (ecstasy) pharmacokinetics in a CYP2D6 poor metaboliser and in nine CYP2D6 extensive metabolisers. *Eur J Clin Pharmacol* 2005; **61**:551–554.
10 Meyer MR, Peters FT, Maurer HH. The role of human hepatic cytochrome P450 isozymes in the metabolism of racemic 3,4-methylenedioxy-methamphetamine and its enantiomers. *Drug Metab Dispos* 2008; **36**:2345–2354.
11 Gilhooly TC, Daly AK. CYP2D6 deficiency, a factor in ecstasy related deaths? *Br J Clin Pharmacol* 2002; **54**:69–70.
12 Yubero-Lahoz S, Pardo R, Farre M, Mathuna BÓ, Torrens M, Mustata C, *et al*. Changes in CYP1A2 activity in humans after 3,4-methylene-dioxymethamphetamine (MDMA, ecstasy) administration using caffeine as a probe drug. *Drug Metab Pharmacokinet* 2012; **27**:605–613.