Supplementary Material
CYP2D6 function moderates the pharmacokinetics and pharmacodynamics of MDMA in a controlled study in healthy subjects

Yasmin Schmid,[1] Patrick Vizeli,[1] Cédric M. Hysek,[1], Katharina Prestin[2], Henriette E. Meyer zu Schwabedissen,[2] Matthias E. Liechti[1]

[1]Division of Clinical Pharmacology and Toxicology, Department of Biomedicine and Department of Clinical Research, University Hospital Basel, Basel, Switzerland
[2]Department of Pharmaceutical Sciences, University of Basel, Basel, Switzerland

Correspondence: Prof. Dr. Matthias E. Liechti, Division of Clinical Pharmacology and Toxicology, University Hospital Basel, Hebelstrasse 2, Basel, CH-4031, Switzerland; Tel: +41 61 328 68 68; Fax: +41 61 265 45 60; E-mail: matthias.liechti@usb.ch

Materials and methods

Study hypothesis

The primary hypothesis was that absence of CYP2D6 activity in PMs and decreased activity in IMs would lower the CYP2D6-mediated conversion of MDMA to HMMA compared with CYP2D6 EMs, thus increasing the concentrations of MDMA and MDA and toxicodynamic effects of MDMA.

Study design

This was a prospectively designed pooled analysis of eight double-blind, placebo-controlled, crossover studies in healthy subjects [1-8] including a total of 142 subjects. The prespecified primary endpoint of the pooled analysis was to assess the effects of polymorphisms in CYP2D6 on the PK of MDMA in all the 142 participants in all of the studies. In seven studies each including 16 subjects, a total of 112 subjects received MDMA at a dose of 125 mg, placebo, one of eight pretreatments plus MDMA, or the pretreatment alone [1-6, 8]. In one study, 30 subjects received MDMA at a dose of 75 mg, placebo, or methylphenidate [7]. Washout periods between treatment periods were at least 7 days. Only data after the administration of MDMA alone without other treatments were included in this analysis and the washout was considered sufficiently long to exclude any effects of the other treatments on the effects of MDMA alone. All of the studies were registered at ClinicalTrials.gov (NCT00886886, NCT00990067, NCT01136278, NCT01270672, NCT013861177, NCT01465685, and NCT01771874). All of the studies were approved by the local ethics committee (Ethikkommission Basel) and the Swiss Agency for Therapeutic Products (Swissmedic) and conducted in accordance with the Declaration of Helsinki. The administration of MDMA in healthy subjects was authorized by the Swiss Federal Office for Public Health (BAG), Bern, Switzerland. Informed consent was obtained from all participants included in the studies.

Subjects

A total of 142 healthy European/Caucasian subjects, aged 18-45 years, were recruited from the University of Basel campus and participated in the study. One genotyping sample was missing, one participant did not give consent for genotyping, and a full concentration-time profile could not be obtained in one participant, resulting in data from 139 participants (69 male, 70 female, mean age ± SD: 24.9 ± 4.1 years; range: 18-44 years) that were included in the analysis. A total of 110 subjects (54 male, 56 female) received 125 mg MDMA (mean ± SD: 1.9 ± 0.3 mg/kg), and 29 subjects (15 male, 14 female) received 75 mg MDMA (1.1 ± 0.1 mg/kg) [9, 2-4]. The exclusion criteria were a history of psychiatric disorders, physical illness, a lifetime history of using illicit drugs more than five times (with the exception of past cannabis use), illicit drug use within the last 2 months, illicit drug use during the study, determined by urine tests that were conducted before the test sessions, and the use of drugs that interact with CYP function. The detailed exclusion criteria were reported elsewhere [9, 2-4].

Study drug

(±)MDMA hydrochloride (Lipomed AG, Arlesheim, Switzerland) was administered orally in a single dose of 125 or 75 mg. Similar doses are found in ecstasy pills [10] and have been used in clinical studies [11]. The dose range was 0.8-2.7 mg/kg (mean = 1.7 mg/kg).

Blood sampling and drug analysis

Blood samples were collected in lithium heparin tubes 0, 0.33, 0.67, 1, 1.5, 2, 2.5, 3, 4, and 6 h after administration of MDMA or placebo and immediately centrifuged. Plasma was stored at -20°C until analysis. Plasma concentrations of MDMA, MDA, and HMMA were determined as previously described [2, 5]. HMMA concentrations were determined twice after enzymatic deglucuronidation in 76 subjects and as unconjugated HMMA levels in 124 of the 139 subjects. Both HMMA concentrations (unconjugated and total after deglucuronidation) were reported. The lower limit of quantification concentrations were 1 ng/ml for all analytes [2].

Pharmacodynamic measures

Blood pressure, heart rate, body temperature, and subjective drug effects were assessed repeatedly before and 0, 0.33, 0.67, 1, 1.5, 2, 2.5, 3, 4, 5, and 6 h after MDMA or placebo administration as previously described [9, 1]. Specifically, heart rate and systolic and diastolic blood pressure were

measured by an OMRON M7 blood pressure monitor (OMRON Healthcare Europe NA, Hoofddorp, The Netherlands) in the dominant arm after a resting time of 5-10 min with the volunteer sitting in bed with the back supported. Measures were taken twice per time-point with an interval of 1 min, and the average was used for analysis. Between measures subjects were allowed to engage in non-strenuous activities. Core (tympanic) temperature was assessed using a GENIUS^{TM} 2 ear thermometer (Tyco Healthcare Group LP, Watertown, NY, USA). Subjective measures included Visual Analogue Scales (VAS). VAS included: "any drug effect" and "drug liking". VAS were presented as 100 mm horizontal lines marked with "not at all" on the left and "extremely" on the right. Additional VASs were included in the individual studies.

DNA extraction and genotyping

Genomic DNA was extracted from whole blood using the QIAamp DNA Blood Mini Kit (Qiagen, Hombrechtikon, Switzerland) and automated QIAcube system. Genotyping was performed using commercial TaqMan SNP genotyping assays (LuBio Science, Lucerne, Switzerland).

Alleles associated with normal (*2), reduced (*9, *10, *17, *29, and *41), no (*3, *4, *5, and *6), or enhanced CYP2D6 activity (*xN) were classified according to previous studies [12-14]. CYP2D6*3 (rs35742686, 2549delA, assay:C_32407232_50), CYP2D6*4 (rs3892097, 184G>A, assay:C_27102431_D0, and rs1065852, 100C>T, assay:C_11484460_40), CYP2D6*6 (rs5030655, 1707delT, assay:C_32407243_20), CYP2D6*9 (rs72549350, 2613-2615delAGA, assay:Hs00225796_CE), CYP2D6*10 (rs1065852, 100C>T, assay:C_11484460_40), CYP2D6*17 (rs28371706, 1023C>T, assay: C_2222771_A0, and rs16947, 2850C>T), CYP2D6*29 (rs59421388, 3183G>A, assay: C_3486113_20), and CYP2D6*41 (rs28371725, 2988G>A, assay: C_34816116_20, and rs16947, 2850C>T, assay: C_27102425_10). CYP2D6*2 was defined as rs16947, 2850C>T, assay: C_27102425_10 with no alterations in rs28371706 or rs28371725. CYP2D6 gene deletion (allele *5) and duplication/multiplication (allele *xN) were determined using the TaqMan Copy Number Assay (Hs00010001_cn and Hs04502391_cn).

CYP2D6 Phenotyping

CYP2D6 activity was determined 14 days before or after the administration of MDMA using the dextromethorphan (DM) / dextrorphan (DX) ratio in urine collected over 8 h after oral administration of DM (25 mg) [15, 16]. Poor-metabolizer were defined as DM/DX ≥ 0.3. Intermediate-metabolizer function was defined as 0.1 ≤ DM/DX < 0.3. Extensive metabolizer function was defined as ≤ DM/DX < 0.1. We did not define UMs by phenotyping because these cannot be well separated from EMs [15, 16] and because only two subjects were genotyped as UMs. The results of the phenotyping and genotyping were not known to the subjects or investigators during the conduct of the study.

Pharmacokinetic analyses

The plasma concentration data were analyzed using non-compartmental methods using Phoenix WinNonlin 6.4 (Certara, Princeton, NJ, USA). Peak plasma concentrations (C_{max}) and the time to reach C_{max} (T_{max}) were obtained directly from the observed data. The terminal elimination rate constant (λ_z) for MDMA and HMMA was estimated by log-linear regression after semilogarithmic transformation of the data using at least three data points of the terminal linear phase of the concentration-time curve. The terminal elimination half-life ($t_{1/2}$) was calculated using λ_z and the equation $t_{1/2} = \ln_2/\lambda_z$. The area under the concentration-time curve (AUC) from 0 to 6 h after dosing (AUC_6) was calculated using the linear trapezoidal method. Plasma concentrations were only determined (shown) up to 6 h after MDMA administration because the aim of the study was to assess potential changes in MDMA plasma levels while relevant pharmacodynamics effects or MDMA are present. Effects of MDMA last only 6 hrs despite its longer presence in plasma due to acute tolerance, possibly because of monoamine depletion, resulting in no effect until monoamine stores are refilled [1, 2]. It was not possible to determine $t_{1/2}$ for MDA because of its long $t_{1/2}$, which would require sampling for an extended time.

Statistical analyses

The statistical analyses were performed using Statistica 12 software (StatSoft, Tulsa, OK, USA). Group differences were analyzed using one-way analysis of variance (ANOVA), with genotype, enzyme activity score, CYP2D6 phenotype, and sex as between-subjects group factors, followed by the Tukey *post hoc* test. To account for differences in body weight and dosing, plasma levels were dose-normalized to the mean dose of MDMA per actual body weight (1.7 mg/kg), and the mg/kg dose

of MDMA was included as a covariate in the analysis of pharmacodynamic effects. Data from the 75 and 125 mg dose groups were analyzed together. Sensitivity analysis of the 125 mg dose subgroup alone revealed results that were similar to the analyses of the total sample. Adding, the eight pooled studies as covariate to the analyses yielded no interactions with the effects of the polymorphisms confirming the absence of confounding by study. Previous experience with MDMA or total illicit substances (excluding cannabis) did not moderate any of the outcomes. Because there were only two UMs in the present study and because the group was too small to be statistically characterized, the two UMs were included in the EM group with an activity score = 2. Spearman rank correlations were used to assess associations between variables.

Additional results

CYP2D6 phenotype-genotype concordance: Six of the seven genetically determined PMs were phenotyped as PMs while one (*3/*3) was phenotyped as EM. Eight of the 19 genetically determined IMs were phenotyped as IMs, and 11 were phenotyped as EMs. Of the 113 subjects who were genotyped as EMs, 99 were congruently phenotyped as EMs, 11 were phenotyped as IMs, and three (*2/*4, *1/*4, *2/*5) were phenotyped as PMs (**Table S1**). The DM/DX ratio correlated with the MDMA/HMMA C_{max} ratio and MDMA/HMMA AUC_6 ratio (R_s = 0.52 and 0.52, respectively, both P < 0.001, N = 76), indicating that both of these ratios consistently reflected CYP2D6 activity.

Concentration-effect relationships: We tested whether differences in plasma concentrations of MDMA were linked to pharmacodynamic effects. Subjects with higher plasma MDMA levels presented greater cardiovascular and subjective effects. The elevations in systolic blood pressure at 1 h correlated with MDMA plasma concentrations at 1 h (C_1)(R_s = 0.60, P < 0.001, N = 139) across subjects. Any drug effect at 1 h correlated with MDMA C_1 (R_s = 0.50, P < 0.001). Similarly, drug liking at 1 h correlated with MDMA C_1 (R_s = 0.33, P < 0.001).

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. The MDMA/HMMA AUC_6 ratio correlated with the AUC values for any drug effect (R_s = 0.44, P < 0.001) and drug liking (R_s = 0.43, P < 0.001), indicating greater overall psychotropic effects in subjects with lower CYP2D6 function. Similar associations were also present between the MDMA/unconjugated HMMA ratio and MDMA effects.

Supplementary Table

Table S1. Effect of CYP2D6 activity on the pharmacokinetics of MDMA (n=139)

CYP2D6 genotype	PM	IM	EM	EM	EM	UM	$F_{4,134}$ =	P =
Activity score	0	0.5	1	1.5	2	3		
subjects (n, total n=139)	7	19	33	17	61	2		
subjects with lower dose (n, n=29)	0	11	3	7	8	0		
Alleles (n)	*4/*4(5) *3/*3(1) *3/*5(1)	*4/*10(6) *4/*41(4) *5/*10(2) *5/*41(1) *10/*41(4)[a] *10/*10(1)[a] *4/*9(1)	*1/*4(11) *1/*3(2) *1/*6(2) *2/*4(15) *2/*5(1) *9/*10(2)	*1/*41(4) *1/*10(2) *1/*9(3) *2/*10(3) *2/*41(5)	*1/*1(22) *1/*2(23) *2/*2(16)	*1/*2 (x2)(2)		
CYP2D6 phenotype	PM (6) EM (1)	IM (8) EM (11)	EM (28) IM (2) PM (3)	EM (15) IM (2)	EM (54) IM (7)	EM (2)		
Dextromethorphan / dextrorphan	1.0±0.53	0.10±0.02***	0.25±0.12***	0.04±0.01***	0.04±0.01***		8.26	<0.001
Women (n, %)	3 (43)	8 (42)	17 (52)	12 (71)	29 (46)		0.87	0.49
Age	25.4±1.2	23.7±0.8	25.6±0.9	23.4±0.9	25.3±0.5		1.46	0.22
MDMA expirience (n, %)	3 (43)	4 (21)	5 (15)	4 (24)	12 (19)		0.72	0.58
Any substance expirience (n, %)	4 (57)	5 (26)	14 (42)	7 (41)	21 (33)		0.75	0.56
MDMA C_{max} (ng/ml)	235±13	220±10	218±5	207±7	197±4*		4.45	0.002
MDMA T_{max} (h)	2.2±0.2	2.6±0.2	2.6±0.2	2.6±0.2	2.5±0.1		0.29	0.88
MDMA AUC_6 (ng·h/ml)	1032±56	922±42	928±25	872±35	823±16**		5.59	<0.001
MDMA $t_{1/2}$ (h)	8.5±1.9	7.8±0.6	8.1±0.6	6.4±0.4	7.9±0.4		0.55	0.70
MDMA $C_{0.3}$	8.3±3.3	30±18	9.6±3.5+	7.3±3.1	5.5±1.9++		3.09	0.019
MDMA $C_{0.6}$	92.9±8.9	33.0±10*	58.0±8.7	46.5±12	42.4±5.3*		3.05	0.019
MDMA C_1	146±11	93±15	120±10	104±14	90±6.4		2.96	0.022
MDMA $C_{1.5}$	202±13	156±17	168±9.6	157±14	143±6.5		2.46	0.049
MDMA C_2	216±7.5	184±14	191±7.4	187±13	169±4.6*		2.99	0.021
MDMA $C_{2.5}$	232±13	211±12	196±6.7	191±12	182±3.8**		4.87	0.001
MDMA C_3	219±13	201±10	195±6.1	183±7.7	180±4.1*		3.23	0.014
MDMA C_4	187±15	192±8.1	185±5.9	174±5.9	171±4.1		2.19	0.074
MDMA C_6	164±13	155±5.5	152±4.4	142±5.9	141±4.2		2.13	0.081
MDA C_{max}	18.0±3.9	9.6±0.5***	12.2±1.3*	11.5±0.7*	10.2±0.3***		5.53	<0.001
MDA AUC_6	61.8±8.2	39.9±2.1***	44.8±2.8**	44.9±2.7*	40.0±1.3***		5.73	<0.001
MDA T_{max}	4.6±0.6	5.7±0.4	5.7±0.2	5.3±0.3	5.9±0.2		1.88	0.12
MDMA AUC_6/MDA AUC_6	20.3±5.5	24.8±2.3	23.2±1.4	20.3±1.4	22.2±1.0		0.88	0.48
HMMA C_{max}	27.4±9.6	69.6±10	80.4±20	84.4±11	128±12+		[b]4.00	<0.01
HMMA AUC_6	118±38	266±31	363±91	427±64	571±55++		[b]4.44	<0.01
HMMA T_{max}	5.0±1.0	3.8±0.3	3.5±0.3	3.3±0.3	2.7±0.2*+		[b]4.96	<0.01
HMMA $t_{1/2}$	8.2	10.9±1.5	10.3±1.0	9.6±1.0	7.9±0.4		[c]2.40	0.06
MDMA C_{max} / HMMA C_{max}	11.3±5.4	4.5±0.8**	4.2±0.6**	3.7±1.1**	2.1±0.2***+		[b]7.91	<0.001
MDMA AUC_6/HMMA AUC_6	10.9±4.4	4.6±0.8*	3.8±0.5**	3.8±1.4**	2.0±0.2***+		[b]6.97	<0.001
MDMA/HMMA $C_{1.5}$	15.1±2.6	6.0±1.4**	4.1±0.6**	4.2±1.7**	2.0±0.2***++		[b]8.67	<0.001
MDMA/HMMA C_2	12.9±3.8	5.8±1.4	4.4±0.6*	4.8±1.7*	2.0±0.2***++		[b]6.98	<0.001
MDMA/HMMA C_3	10.7±4.6	5.1±1.2	4.2±0.6	4.2±1.5	2.1±0.3**+		[b]5.11	<0.01
MDMA/HMMA C_4	8.7±3.6	4.6±1.0	3.9±0.7	4.1±1.4	2.2±0.3*		[b]3.98	<0.01
MDMA/HMMA C_6	9.9±5.0	3.5±0.6**	3.4±0.5**	3.6±1.0**	2.3±0.3***		[b]5.60	<0.001

*P<0.05, **P<0.01, ***P<0.001 compared with PMs. +P<0.05, ++P<0.01, +++P<0.001 compared with IMs. [a]some investigators would consider these combinations as EM. PM, poor metabolizer; IM, intermediate metabolizer; EM, extensive metabolizer; C, plasma concentration at given time point; C_{max} maximum plasma concentration, AUC, area under the plasma concentration-time curve; AUC_6, AUC from time 0-6h; $t_{1/2}$, plasma half-life; T_{max}, time to reach C_{max}. Values are means±SEM. [b]F4,71 (N=76; 2 PMs, 15 IMs, 14 EMs 1.0, 12 EMs 1.5, 33 EMs 2.0 and UMs). [c]F4,64 (N=69, 1 PM, 11 IMs, 13 EMs 1.0, 11 EMs 1.5, 33 EMs 2.0 and UMs).

Table S2. Effect of CYP2D6 activity on unconjugated HMMA formation (N=124).

CYP2D6 genotype	PM	IM	EM	EM	EM	UM	$F_{4,119}$ =	P =
Activity score	0	0.5	1	1.5	2	3		
subjects (n, total n=124)	4	18	30	15	56	1		
HMMA unconj. C_{max}	2.9±1.0	4.2±0.8	4.3±0.5	6.9±0.9	6.8±0.4++		6.93	<0.001
HMMA unconj. AUC_6	11.9±4.0	17.7±2.8	17.7±2.1	29.2±3.9	28.9±1.5++		7.45	<0.001
HMMA unconj. T_{max}	2.6±0.2	2.4±0.3	2.0±0.1	1.95±0.3	1.59±0.1++		5.02	<0.001
HMMA unconj. $t_{1/2}$	9.0±0.8	9.6±1.7	7.6±0.8	6.6±0.5	7.7±0.5		1.13	0.35
MDMA C_{max} / HMMA unconj. C_{max}	124±65	81±15	65.0±6.8*	43.7 ±9.7**	35.1±2.6***+++		9.46	<0.001
MDMA AUC_6/HMMA unconj. AUC_6	129±59	78.5±15	70.5±8.4	44.7±11	35.1±3.2***++		8.69	<0.001
MDMA/HMMA unconj. $C_{1.5}$	136±58	64±14**	52±6.7***	38±11***	26±2.2***++		11.09	<0.001
MDMA/HMMA unconj. C_2	142±66	76±17*	60±6.8**	43±10***	33±2.8***++		9.51	<0.001
MDMA/HMMA unconj. C_3	122±58	83±15	68±6.9	48±11*	39±2.9**++		8.1	<0.001
MDMA/HMMA unconj. C_4	108±47	84±15	71±7.4	51±10	44±3.5*++		6.18	<0.001
MDMA/HMMA unconj. C_6	113±49	76±12	72±9.8	52±10*	43±2.7**+		6.12	<0.001

*P<0.05, **P<0.01, ***P<0.001 compared with PMs. +P<0.05, ++P<0.01, +++P<0.001 compared with IMs. PM, poor metabolizer; IM, intermediate metabolizer; EM, extensive metabolizer; C, plasma concentration at given time point; C_{max} maximum plasma concentration, AUC, area under the plasma concentration-time curve; AUC_6, AUC from time 0-6h; $t_{1/2}$, plasma half-life; T_{max}, time to reach C_{max}. Values are means±SEM.

Supplementary Figures

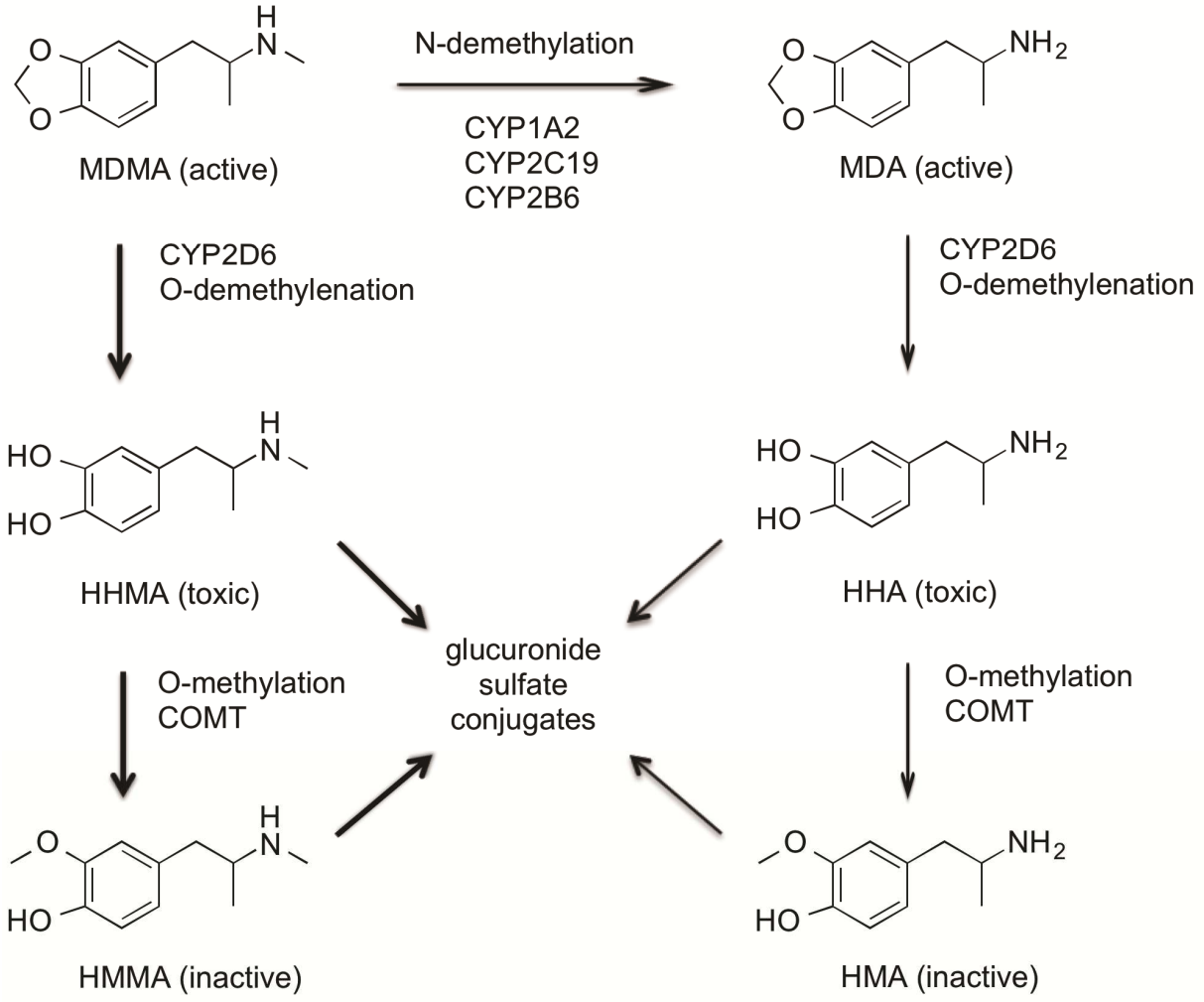

Figure S1. Metabolism of MDMA in humans. MDMA is O-demethylenated primarily by CYP2D6 to 3,4-dihydroxymethamphetamine (HHMA), which is then O-methylated to 4-hydroxy-3-methoxymethamphetamine (HMMA) by catechol-O-methyltransferase (COMT) [17-20], the main inactive metabolite of MDMA in humans [21, 22]. Additionally, MDMA is N-demethylated mainly by CYP1A2 and CYP2B6 and to a lesser extent CYP3A4 and CYP2C19 [17, 20] to the minor active metabolite 3,4-methylenedioxyamphetamine (MDA) [2, 21, 23]. MDA is O-demethylenated by CYP2D6 to 3,4-dihydroxyamphetamine (HHA), which is then O-methylated by COMT to 4-hydroxy-3-methoxy-amphetamine (HMA) [17].

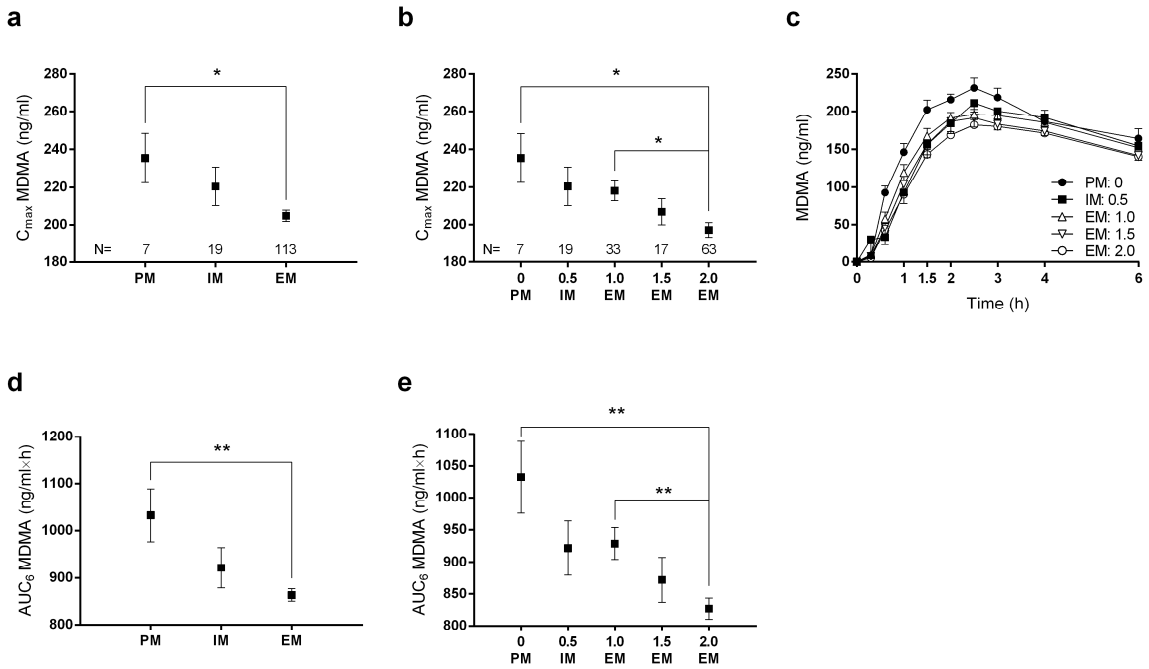

Figure S2. Effect of CYP2D6 on the PK of MDMA. (**a**, **b**) C_{max} values of MDMA for different (**a**) CYP2D6 phenotypes predicted based on genotyping and (**b**) CYP2D6 activity score groups based on genotyping. (**c**) Plasma concentration-time curves of MDMA for different CYP2D6 activity score groups. (**d, e**) AUC_6 values of MDMA for different (**d**) CYP2D6 genotypes and (**e**) CYP2D6 activity score groups (**e**). Plasma MDMA levels were higher in the activity score 0 group (PMs) compared with other activity scores up to 3 h **(c)**. MDMA C_{max} and AUC_6 values were higher in PMs compared with the EM genotype (**a**, **d**) and the activity score 2 group (**b, e**). Within EMs, the activity score 1 group presented higher plasma levels of MDMA compared with the activity score 2 group **(b**, **e**). The data are expressed as mean ± SEM. The number of subjects per group is indicated below each value in (**a**) and (**b**). *$P < 0.05$, **$P < 0.01$.

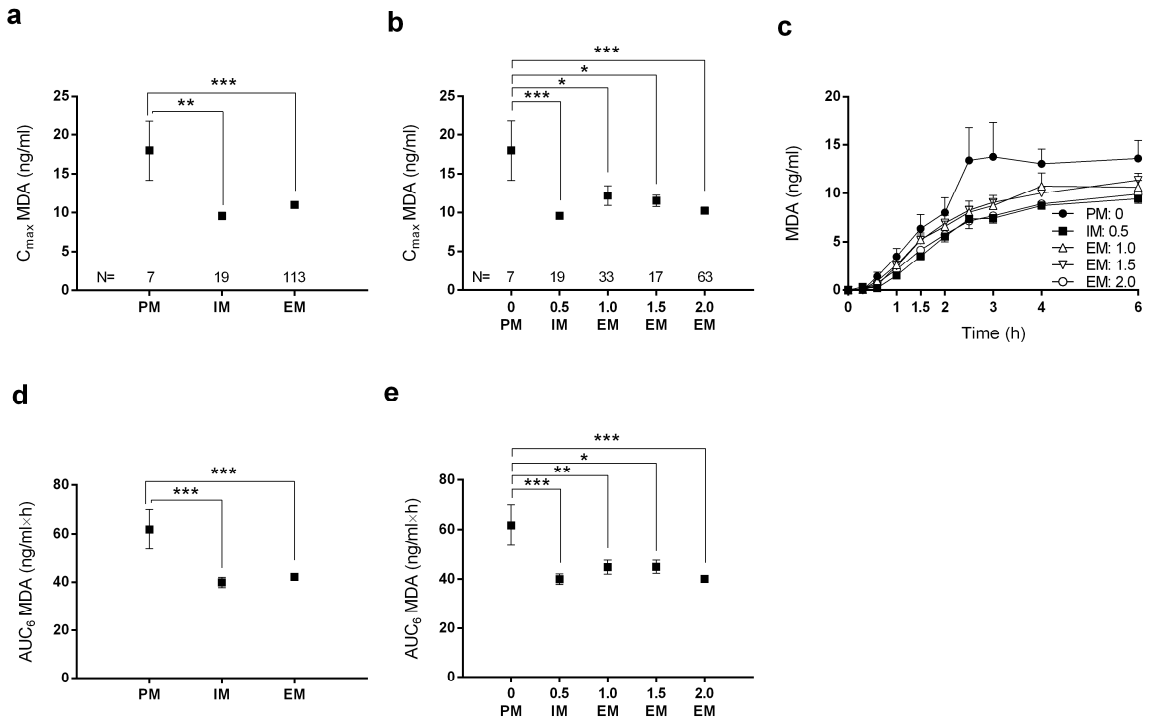

Figure S3. Effects of predicted CYP2D6 phenotypes based on genotyping on the PK of MDA. (**a**, **b**) C_{max} values of MDA for different (**a**) CYP2D6 predicted phenotypes and (**b**) genetically predicted CYP2D6 activity score groups (**b**). (**c**) Plasma concentration-time curves of MDA for different CYP2D6 activity score groups. (**d**, **e**) AUC_6 values of MDA for different (**d**) CYP2D6 genotypes and (**e**) CYP2D6 activity score groups. C_{max} and AUC_6 values of MDA were higher in PMs compared with IMs or EMs and compared with all lower activity score groups. The data are expressed as mean ± SEM. The number of subjects per group is indicated below each value in (**a**) and (**b**). **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

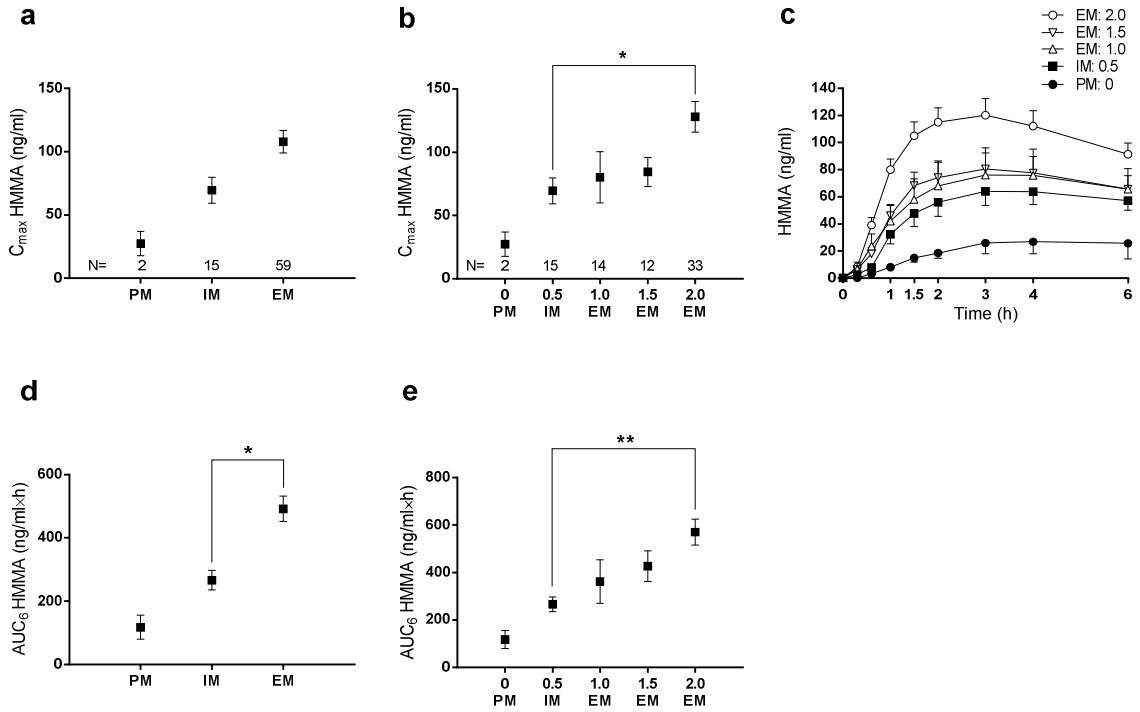

Figure S4. Effects of predicted CYP2D6 phenotypes based on genotyping on the PK of HMMA. (**a**, **b**) C_{max} values of HMMA for different (**a**) CYP2D6 genotypes and (**b**) genetically determined CYP2D6 activity score groups. (**c**) Plasma concentration-time curves of HMMA for different CYP2D6 activity score groups. (**d**, **e**) AUC_6 values of HMMA for different (**d**) CYP2D6 genotypes and (**e**) CYP2D6 activity score groups. C_{max} and AUC_6 levels of HMMA were lower in subjects with lower CYP2D6 function. Because the number of PMs was small, differences between PMs and the other groups did not reach significance. The data are expressed as mean ± SEM. The number of subjects per group is indicated below each value in (**a**) and (**b**). *P < 0.05, **P < 0.01.

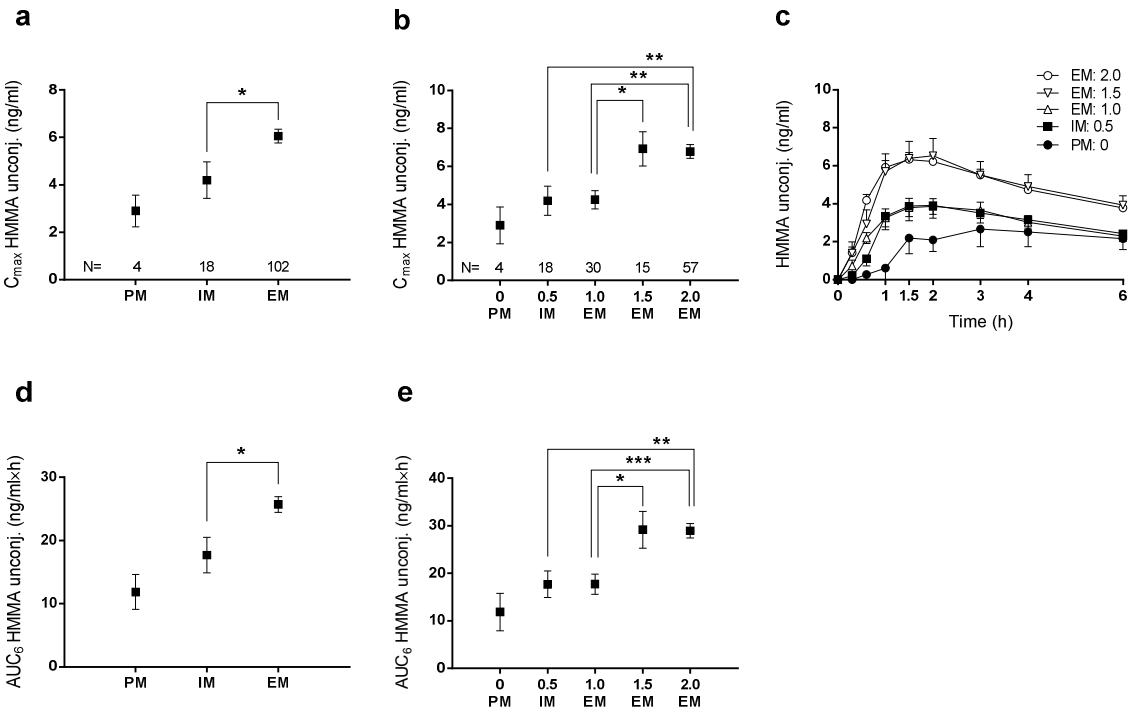

Figure S5. Effects of predicted CYP2D6 phenotypes based on genotyping on the PK of unconjugated HMMA. (**a**, **b**) C_{max} values of HMMA for different (**a**) CYP2D6 genotypes and (**b**) genetically determined CYP2D6 activity score groups. (**c**) Plasma concentration-time curves of HMMA for different CYP2D6 activity score groups. (**d**, **e**) AUC_6 values of HMMA for different (**d**) CYP2D6 genotypes and (**e**) CYP2D6 activity score groups. C_{max} and AUC_6 levels of HMMA were lower in subjects with lower CYP2D6 function. Because the number of PMs was small, differences between PMs and the other groups did not reach significance. The data are expressed as mean ± SEM. The number of subjects per group is indicated below each value in (**a**) and (**b**). **P* < 0.05, ***P* < 0.01, ****P* < 0.001. HMMA indicates unconjugated HMMA concentrations.

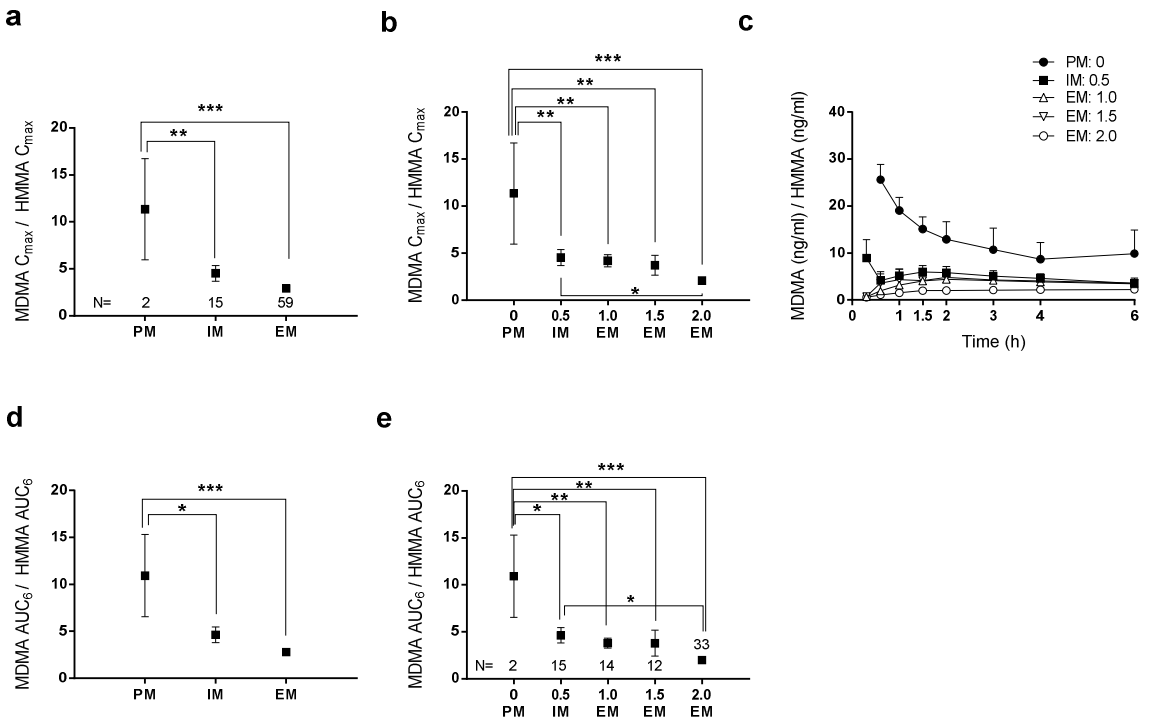

Figure S6. Effects of predicted CYP2D6 phenotypes based on genotyping on the conversion of MDMA to HMMA (MDMA AUC_6 / HMMA AUC_6 ratio). (**a**, **b**) MDMA C_{max} / HMMA C_{max} ratios for different (**a**) CYP2D6 predicted phenotypes and (**b**) CYP2D6 predicted activity score groups. (**c**) Changes in MDMA-to-HMMA bioconversion over time for different CYP2D6 activity score groups. (**d**, **e**) MDMA AUC_6 / HMMA AUC_6 ratios for different (**d**) CYP2D6 genotypes and (**e**) CYP2D6 activity score groups. MDMA-to-HMMA ratios were lower in EMs compared with IMs and PMs, indicating higher MDMA-to-HMMA conversion in subjects with higher CYP2D6 function. The data are expressed as mean ± SEM. The number of subjects per group is indicated below each value in (**a**) and (**e**). **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

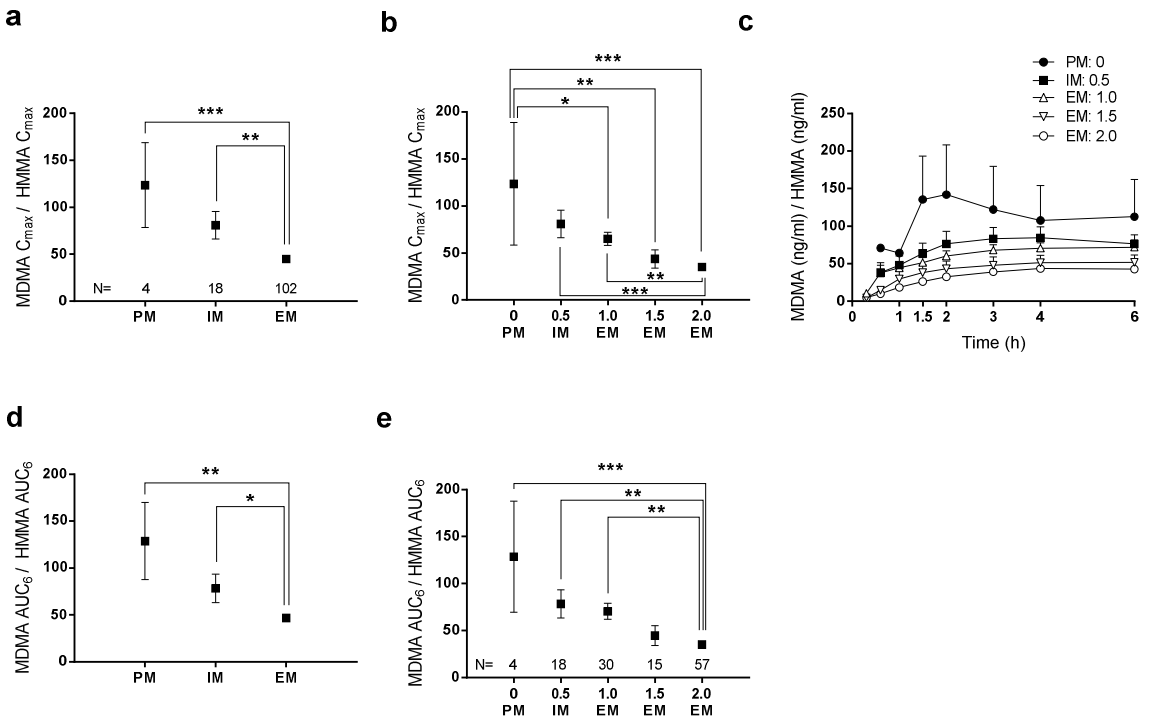

Figure S7. Effects of predicted CYP2D6 phenotypes based on genotyping on the conversion of MDMA to unconjugated HMMA (MDMA AUC_6 / HMMA AUC_6 ratio). (**a**, **b**) MDMA C_{max} / HMMA C_{max} ratios for different (**a**) CYP2D6 predicted phenotypes and (**b**) CYP2D6 predicted activity score groups. (**c**) Changes in MDMA-to-HMMA bioconversion over time for different CYP2D6 activity score groups. (**d**, **e**) MDMA AUC_6 / HMMA AUC_6 ratios for different (**d**) CYP2D6 genotypes and (**e**) CYP2D6 activity score groups. MDMA-to-HMMA ratios were lower in EMs compared with IMs and PMs, indicating higher MDMA-to-HMMA conversion in subjects with higher CYP2D6 function. The data are expressed as mean ± SEM. The number of subjects per group is indicated below each value in (**a**) and (**e**). **P* < 0.05, ***P* < 0.01, ****P* < 0.001. HMMA indicates unconjugated HMMA concentrations.

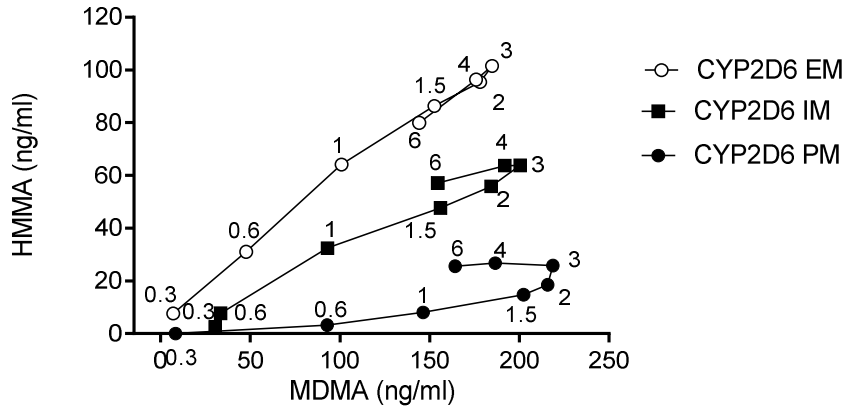

Figure S8. Hysteresis plot showing the biotransformation of MDMA to HMMA in the different CYP2D6 phenotypes based on genotyping. The mean MDMA *vs*. HMMA plasma concentration profiles demonstrated three distinct curves for CYP2D6 EMs, IMs, and PMs. HMMA was formed to a lesser extent in subjects with reduced CYP2D6 function. HMMA concentrations were determined after enzymatic deglucuronidation. The data are expressed as means in two PMs, 15 IMs, and 59 EMs.

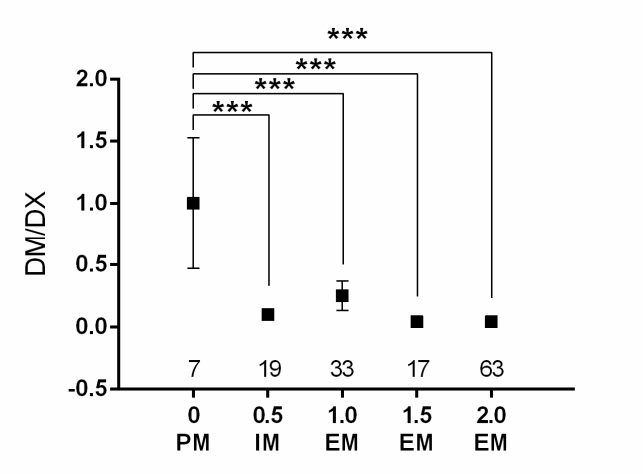

Figure S9. Dextromethorphan (DM)/dextrorphan (DX) ratio plotted against the genetically derived CYP2D6 activity score groups. The DM/DX ratio was significantly greater in subjects with a CYP2D6 activity score of 0 (genetically defined PMs) compared with subjects with higher activity scores (***P < 0.001). DM/DX ratios did not differ across activity scores of 0.5 to 3.0 (two subjects with a score of 3 = UMs were included in the 2.0 = EM group). The number of subjects in each group and the allelic distribution are shown in Table 1. The data are expressed as mean ± SEM. The number of subjects per group is shown below each value.

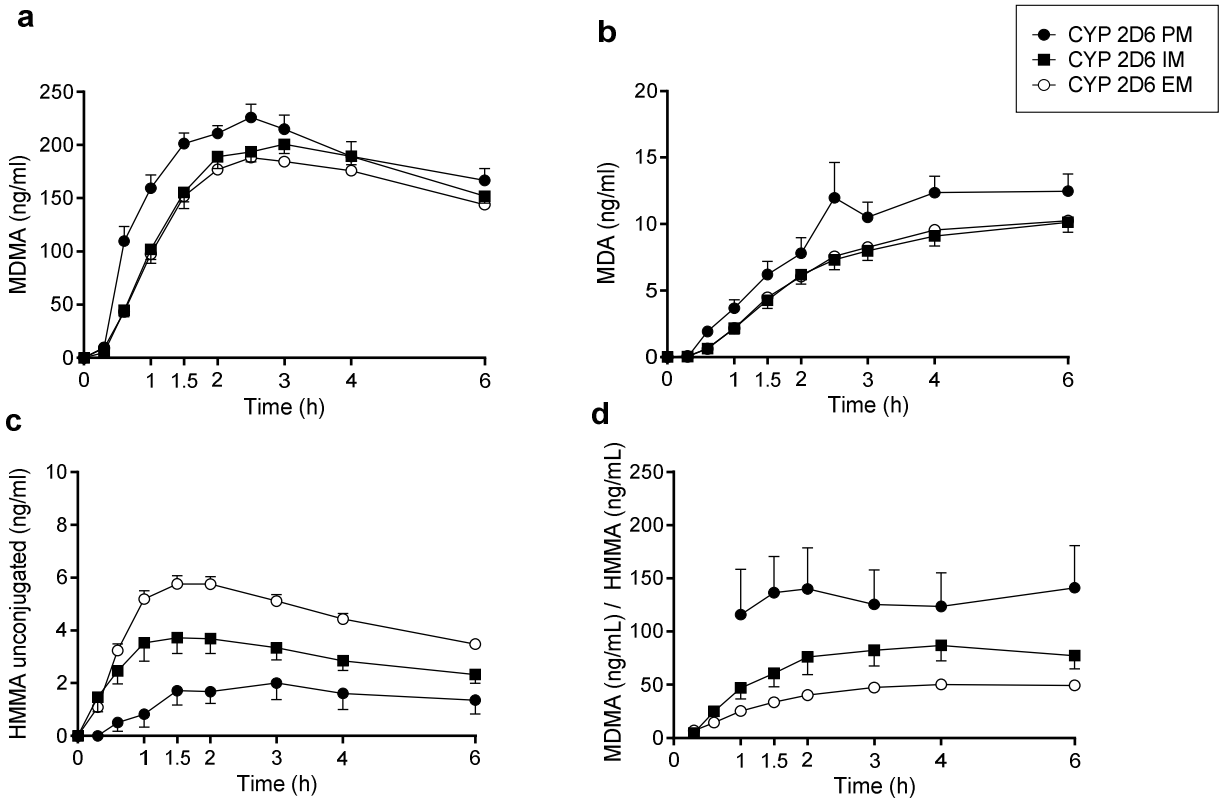

Figure S10. The CYP2D6 phenotype derived from phenotyping using dextromethorphan moderated the pharmacokinetics of (**a**) MDMA, (**b**) MDA, and (**c**) unconjugated HMMA and (**d**) the MDMA/HMMA ratio similar to the CYP2D6 phenotypes based on genotyping shown in the main article (Fig. 1). Lower CYP2D6 function as in PMs resulted in (**a**) higher MDMA and (**b**) higher MDA but (**c**) lower HMMA plasma levels and (**d**) lower MDMA/HMMA ratios compared with higher CYP2D6 function as in EMs. CYP2D6 phenotype altered the C_{max} of MDMA ($F_{2,136}$ = 5.22, P = 0.007), with PMs showing a higher C_{max} (236 ± 10 ng/ml) compared with EMs (204 ± 3 ng/ml, P = 0.017). CYP2D6 phenotype altered the AUC_6 of MDMA ($F_{2,136}$ = 7.45, P < 0.001), with PMs showing higher AUC_6 values (1041 ± 49 ng/ml) compared with EMs (860 ± 14 ng/ml, P < 0.001) (**a**). CYP2D6 PMs exhibited higher AUC_6 levels of MDA (56 ± 6 ng/ml) compared with IMs (39 ± 3 ng/ml) and EMs (42 ± 1 ng/ml; P = 0.004 and 0.001, respectively) (**b**). CYP2D6 phenotype moderated the C_{max} of HMMA ($F_{2,121}$ = 8.559, P < 0.001), and C_{max} values were higher in EMs (6.2 ± 0.3 ng/ml) compared with IMs (4.2 ± 0.6 ng/ml) and PMs (2.2 ± 0.7 ng/ml; P = 0.024 and P < 0.001, respectively). CYP2D6 phenotype influenced the AUC_6 of HMMA ($F_{2.121}$ = 9.84, P < 0.001), with higher values in EMs (26 ± 1 ng/ml) compared with IMs (17 ± 3 ng/ml) and PMs (8.6 ± 3 ng/ml; P = 0.014 and P < 0.001, respectively) (**c**). The T_{max} and $t_{1/2}$ of MDMA and its metabolites did not differ between the different CYP2D6 phenotype groups. CYP2D6 EMs had lower MDMA/HMMA AUC_6 ratios (44 ± 3) compared with IMs (76 ± 15) and PMs (145 ± 34; P = 0.01 and P < 0.001, respectively), and IMs also had lower ratios compared with PMs (P = 0.002), consistent with the differences in the MDMA/HMMA ratios at the different time points after drug administration (**d**). The data are expressed as mean ± SEM. Number of subjects per group: PMs = 9, IMs = 19, EMs = 111 in (**a)** and (**b**) and PMs = 7, IMs = 18, and EMs = 99 in (**c**) and (**d**). MDMA was administered at t = 0 h.

References

1. 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(2):246-55. doi:10.1038/clpt.2011.78.
2. 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.
3. Hysek CM, Brugger R, Simmler LD, Bruggisser M, Donzelli M, Grouzmann E et al. Effects of the alpha$_2$-adrenergic agonist clonidine on the pharmacodynamics and pharmacokinetics of 3,4-methylenedioxymethamphetamine in healthy volunteers. The Journal of pharmacology and experimental therapeutics. 2012;340:286-94.
4. Hysek CM, Schmid Y, Rickli A, Simmler LD, Donzelli M, Grouzmann E et al. Carvedilol inhibits the cardiostimulant and thermogenic effects of MDMA in humans. Br J Pharmacol. 2012;166:2277-88. doi:10.1111/j.1476-5381.2012.01936.x.
5. Hysek CM, Fink AE, Simmler LD, Donzelli M, Grouzmann E, Liechti ME. Alpha-adrenergic receptors contribute to the acute effects of MDMA in humans. Journal of clinical psychopharmacology. 2013;33:658-66.
6. Hysek CM, Simmler LD, Schillinger N, Meyer N, Schmid Y, Donzelli M et al. Pharmacokinetic and pharmacodynamic effects of methylphenidate and MDMA administered alone and in combination. Int J Neuropsychopharmacol. 2014;17:371-81. doi:doi:10.1017/S1461145713001132.
7. Schmid Y, Hysek CM, Simmler LD, Crockett MJ, Quednow BB, Liechti ME. Differential effects of MDMA and methylphenidate on social cognition. J Psychopharmacol. 2014;28:847-56.
8. Schmid Y, Rickli A, Schaffner A, Duthaler U, Grouzmann E, Hysek CM et al. Interactions between bupropion and 3,4-methylenedioxymethamphetamine in healthy subjects. The Journal of pharmacology and experimental therapeutics. 2015;353(1):102-11. doi:10.1124/jpet.114.222356.
9. Hysek CM, Liechti ME. Effects of MDMA alone and after pretreatement with reboxetine, duloxetine, clonidine, carvedilol, and doxazosin on pupillary light reflex. Psychopharmacology. 2012;224:363-76.
10. Brunt TM, Koeter MW, Niesink RJ, van den Brink W. Linking the pharmacological content of ecstasy tablets to the subjective experiences of drug users. Psychopharmacology. 2012;220(4):751-62. doi:10.1007/s00213-011-2529-4.
11. Oehen P, Traber R, Widmer V, Schnyder U. A randomized, controlled pilot study of MDMA (±3,4-methylenedioxymethamphetamine)-assisted psychotherapy for treatment of resistant, chronic post-traumatic stress disorder (PTSD). J Psychopharmacol. 2013;27(1):40-52. doi:10.1177/0269881112464827.
12. Crews KR, Gaedigk A, Dunnenberger HM, Klein TE, Shen DD, Callaghan JT et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for codeine therapy in the context of cytochrome P450 2D6 (CYP2D6) genotype. Clin Pharmacol Ther. 2012;91(2):321-6. doi:10.1038/clpt.2011.287.
13. 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(5):402-8. doi:10.1038/clpt.2013.2.
14. Hicks JK, Bishop JR, Sangkuhl K, Muller DJ, Ji Y, Leckband SG et al. Clinical pharmacogenetics implementation consortium (CPIC) guideline for CYP2D6 and CYP2C19 genotypes and dosing of selective serotonin reuptake inhibitors. Clin Pharmacol Ther. 2015;98(2):127-34. doi:10.1002/cpt.147.
15. Gaedigk A, Simon SD, Pearce RE, Bradford LD, Kennedy MJ, Leeder JS. The CYP2D6 activity score: translating genotype information into a qualitative measure of phenotype. Clin Pharmacol Ther. 2008;83(2):234-42. doi:10.1038/sj.clpt.6100406.
16. Trojan A, Vergopoulos A, Breitenstein U, Seifert B, Rageth C, Joechle W. The discrimiminatory value of CYP2D6 genotyping in predicting the dextromethorphan/dextrorphan phenotype in women with breast cancer. Breast Care. 2012;7:25-31.
17. Kreth KP, Kovar KA, Schwab M, Zanger UM. Identification of the human cytochromes P450 involved in the oxidative metabolism of "Ecstasy"-related designer drugs. Biochem Pharmacol. 2000;59(12):1563-71.
18. 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.

19. Segura M, Farre M, Pichini S, Peiro AM, Roset PN, Ramirez A et al. Contribution of cytochrome P450 2D6 to 3,4-methylenedioxymethamphetamine disposition in humans: use of paroxetine as a metabolic inhibitor probe. Clin Pharmacokinet. 2005;44(6):649-60.
20. 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(11):2345-54. doi:10.1124/dmd.108.021543.
21. Kolbrich EA, Goodwin RS, Gorelick DA, Hayes RJ, Stein EA, Huestis MA. Plasma pharmacokinetics of 3,4-methylenedioxymethamphetamine after controlled oral administration to young adults. Ther Drug Monit. 2008;30(3):320-32.
22. Schindler CW, Thorndike EB, Blough BE, Tella SR, Goldberg SR, Baumann MH. Effects of 3,4-methylenedioxymethamphetamine (MDMA) and its main metabolites on cardiovascular function in conscious rats. Br J Pharmacol. 2014;171(1):83-91. doi:10.1111/bph.12423.
23. de la Torre R, Farre M, Roset PN, Pizarro N, Abanades S, Segura M et al. Human pharmacology of MDMA: pharmacokinetics, metabolism, and disposition. Ther Drug Monit. 2004;26(2):137-44.