Supplement 1

to

Effects of Pharmacokinetic Gene Variation on Therapeutic Drug Levels and Antidepressant Treatment Response

Table 1 Demographic data of patients included in the amitriptyline sample.

	Combined Sample		Wuerzburg Sample		Munich Sample	
	N	**Mean ± SD (range)**	**N**	**Mean ± SD (range)**	**N**	**Mean ± SD (range)**
Included patients	109		62		47	
Age [years]	109	47.51 ± 12.60 (18-78)	62	46.21 ± 12.96 (18-67)	47	49.23 ± 12.04 (27-78)
Male/female	53/56		30/32		23/24	
Non-smoker/Smoker	64/37		38/23		26/14	
Daily number of cigarettes	37	17.89 ± 10.06 (1-55)	23	17.91 ± 10.60 (1-55)	14	17.86 ± 9.55 (5-40)
HAMD Baseline	100	25.56 ± 6.61 (14-46)	59	25.29 ± 7.22 (14-46)	41	25.95 ± 5.67 (16-39)
HAMD Out	108	12.88 ± 7.08 (1-38)	61	12.28 ± 6.79 (2-38)	47	13.66 ± 7.44 (1-35)
Length of disorder [years]	106	14.40 ± 12.43 (0-49)	61	12.79 ± 11.71 (0-46)	45	16.58 ± 13.16 (0-49)
Dose (Out)	109	142.8 ± 72.8 (25-340)	62	115.7 ± 41.4 (25-225)	47	178.6 ± 88.7 (25-340)
Resp/Non-resp (Out)	53/53		36/25		17/28	
Rem/Non-rem (Out)	25/83		18/43		7/40	
Adverse Drug Effects						
NA-None/Mild/ Medium/Severe			54/4/4/0			
Change of antidepressant due to adverse drug effects Yes/No/Missing					1/27/19	
Type			Drowsiness/Sedation Modified Salivation Inner Restlessness Modified Accommodation Weight gain		Drowsiness/Sedation	

N, number of patients; SD, standard deviation; m, male; f, female; HAMD, Hamilton Depression Rating Scale-21; Out, outcome time point; Resp, Response; Rem, Remission

Table 2 Demographic data of patients included in the venlafaxine sample.

	Combined Sample		Wuerzburg Sample		Munich Sample	
	N	**Mean ± SD (range)**	**N**	**Mean ± SD (range)**	**N**	**Mean ± SD (range)**
Included patients	258		130		128	
Age [years]	258	44.52 ± 14.08 (18-75)	130	42.67 ± 13.93 (18-75)	128	46.41 ± 14.03 (19-75)
Male/female	127/131		57/73		70/58	
Non-smoker/Smoker	156/91		80/49		76/42	
Daily number of cigarettes	91	16.90 ± 9.48 (1-50)	49	15.96 ± 8.73 (1-40)	42	17.96 ± 10.25 (1-20)
HAMD Baseline	245	25.74 ± 7.04 (14-46)	123	25.85 ± 7.61 (14-46)	122	25.63 ± 6.44 (14-44)
HAMD Out	254	12.45 ± 6.81 (0-30)	128	9.97 ± 6.26 (0-27)	126	14.97 ± 6.43 (0-30)
Length of disorder [years]	251	12.65 ± 11.19 (0-51)	130	12.47 ± 10.96 (0.25-49)	121	12.84 ± 11.48 (0-51)
Dose (Out)	258	279.8 ± 101.4 (25-525)	130	266.8 ± 99.2 (37.5-450)	128	292.9 ± 102.3 (25-525)
Resp/Non-resp (Out)	123/128		88/40		35/88	
Rem/Non-rem (Out)	75/179		55/73		20/106	
Adverse Drug Effects						
NA-None/Mild/ Medium/Severe			118/6/6/0			
Change of antidepressant due to adverse drug effects Yes/No/Missing					1/78/49	
Type			Drowsiness/Sedation Modified Salivation Inner Restlessness Modified Accommodation Weight gain Cardiovascular Effects Extrapyramidal Effects		Other	

N, number of patients; SD, standard deviation; m, male; f, female; HAMD, Hamilton Depression Rating Scale-21; Out, outcome time point; Resp, Response; Rem, Remission

Table 3 Demographic data of patients included in the mirtazapine sample.

	Combined Sample		Wuerzburg Sample		Munich Sample	
	N	**Mean ± SD (range)**	**N**	**Mean ± SD (range)**	**N**	**Mean ± SD (range)**
Included patients	171		64		107	
Age [years]	171	49.86 ± 14.22 (18-80)	64	48.58 ± 15.13 (18-80)	107	50.63 ± 13.65 (20-80)
Male/female	86/85		32/32		54/53	
Non-smoker/Smoker	102/52		39/25		63/27	
Daily number of cigarettes	52	15.71 ± 7.98 (2-40)	25	14.36 ± 7.15 (2-30)	27	16.96 ± 8.62 (4-40)
HAMD Baseline	153	26.26 ± 6.39 (14-39)	56	27.55 ± 6.84 (14-39)	97	25.52 ± 6.03 (14-396)
HAMD Out	168	12.49 ± 7.67 (0-37)	62	8.87 ± 6.26 (0-24)	106	14.60 ± 7.65 (1-37)
Length of disorder [years]	164	12.15 ± 12.16 (0-48)	64	11.40 ± 10.42 (0-36)	100	12.63 ± 13.19 (0-48)
Dose (Out)	171	45.2 ± 22.9 (7.5-120)	64	35.7 ± 13.0 (7.5-75)	107	50.9 ± 25.5 (7.5-120)
Resp/Non-resp (Out)	79/83		48/14		31/69	
Rem/Non-rem (Out)	52/116		32/30		20/86	
Adverse Drug Effects						
NA-None/Mild/ Medium/Severe			62/1/1/0			
Change of antidepressant due to adverse drug effects Yes/No/Missing					0/38/69	
Type			Drowsiness/Sedation			

N, number of patients; SD, standard deviation; m, male; f, female; HAMD, Hamilton Depression Rating Scale-21; Out, outcome time point; Resp, Response; Rem, Remission

Table 4 Demographic data of patients included in the quetiapine sample.

	Combined Sample		Wuerzburg Sample		Munich Sample	
	N	**Mean ± SD (range)**	**N**	**Mean ± SD (range)**	**N**	**Mean ± SD (range)**
Included patients	193		105		88	
Age [years]	193	46.44 ± 14.09 (18-80)	105	44.97 ± 14.81 (18-80)	88	48.19 ± 13.03 (20-75)
Male/female	98/95		51/54		47/41	
Non-smoker/Smoker	121/69		66/39		55/30	
Daily number of cigarettes	69	16.69 ± 9.45 (1-55)	39	15.97 ± 10.90 (1-55)	30	17.6 ± 7.29 (3-36)
HAMD Baseline	175	26.35 ± 6.74 (14-46)	96	26.41 ± 6.84 (14-46)	79	26.28 ± 6.66 (14-44)
HAMD Out	187	13.26 ± 7.08 (0-33)	103	10.98 ± 6.54 (0-28)	84	16.06 ± 6.73 (2-33)
Length of disorder [years]	186	13.23 ± 11.98 (0-50)	104	16.86 ± 12.10 (0.4-49)	82	13.70 ± 11.89 (0-50)
Dose (Out)	193	211.9 ± 136.6 (12.5-800)	105	216.9 ± 125.7 (25-625)	88	206.0 ± 149.0 (12.5-800)
Resp/Non-resp (Out)	85/100		70/33		15/67	
Rem/Non-rem (Out)	46/141		36/67		10/74	
Adverse Drug Effects						
NA-None/Mild/ Medium/Severe			99/5/1/0			
Change of antidepressant due to adverse drug effects Yes/No/Missing					3/61/24	
Type			Drowsiness/Sedation Cardiovascular Effects Extrapyramidal Effects		Drowsiness Other	

N, number of patients; SD, standard deviation; m, male; f, female; HAMD, Hamilton Depression Rating Scale-21; Out, outcome time point; Resp, Response; Rem, Remission

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Table presenting the combination of the SNPs according to the haplotypes.

	HAPLOTYPE								
UGT2B7		**rs7662029**	**rs7668258**						
	*1	G	C						
	*2	A	T						
ABCB1		**rs1045642**	**rs1128503**	**rs2032582**					
	*1	G	G	C					
	*2	A	A	R					
	*6; *UNK4	A	G	C					
	*8;*16	G	A	C					
	*9;*11	G	A	R					
	*10;*13;*17	G	G	R					
	*18; *UNK3	A	G	R					
CYP2C19		**rs 12248560**	**rs 4244285**	**rs 17878459**	**rs 3758580**				
	*1;*13; *15; e.g.	C	G	G	C				
	*2	C	A	G	T				
	*2C	C	A	C	T				
	*17	T	G	G	C				
CYP2C9		**rs 1057910**	**rs 1799853**						
	*1;*9	A	C						
	*2	A	T						
	*3;*18	C	C						
ABCC2		**rs 2273697**	**rs 3740066**	**rs 717620**					
	*1C	G	T	T					
	*1A/B;*3	G	C	C					
	*2	A	C	C					
	*UNK2	A	T	C					
	*UNK3	G	T	C					
CYP1A2		**rs 2069514**	**rs 762551**						
	*1A	G	C						
	*1F	G	A						
	*1L	A	A						
CYP2D6		**rs 1065852**	**rs 28371725**	**rs 35742686**	**rs 3892097**	**rs 5030655**	**rs 5030656**	**rs 28371720**	**rs 16947**	**rs 1135840**
	*1	G	C	T	C	A	A	A	G	C

*2;*21; *28; e.g.	G	C	T	C	A	A	A	A	G
*3	G	C	D	C	A	A	A	G	C
*4	A	C	T	T	A	A	A	G	G
*5	Complete Gene Deletion								
*9	G	C	T	C	A	D	D	G	C
*10;*17;*37; e.g.	A	C	T	C	A	A	A	G	G
*32;*41	G	T	T	C	A	A	A	A	G
*6	G	C	T	C	D	A	A	G	C

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Number (N) of prescribed psychiatric medication and serum concentration determinations (N(TDM)) at the outcome time point (discharge from the study (Wuerzburg sample) and week 6 (Munich sample)) in the samples.

Psychiatric Drug	N	N(TDM)	Psychiatric Drug	N	N(TDM)
Antidepressants			**Antipsychotics**		
Venlafaxine	353	258	Quetiapine	279	193
Mirtazapine	295	171	Olanzapine	118	66
Trimipramine	158	88	Risperidone	50	24
Amitriptyline	133	109	Aripiprazole	46	23
Citalopram	98	53	Pipamperone	16	1
Escitalopram	80	46	Melperone	11	3
Duloxetine	76	33	Ziprasidone	6	1
Sertraline	52	36	Perazine	6	1
Trazodon	49	31	Amisulpride	5	2
Bupropion	49	21	Haloperidol	4	
Paroxetine	46	24	Clozapine	4	3
Reboxetine	31	8	Paliperidone	1	
Clomipramine	30	18	**Antiepileptics**		
Doxepin	27	19	Lamotrigine	144	75
Nortriptyline	16	14	Pregabaline	45	15
Fluoxetine	7	2	Valproic Acid	27	16
Imipramine	3	1	Gabapentine	24	3
Maprotiline	3	1	Carbamazepine	12	5
Milnacpiran	2	1	Topiramate	5	

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to

Effects of Pharmacokinetic Gene Variation on Therapeutic Drug Levels and Antidepressant Treatment Response

Table 1 Diplotype/phenotype analyses in regard to serum concentrations were performed by Kruskal-Wallis tests. To adjust for alpha-error accumulation, nominal p-values were Bonferroni-corrected for the total number of genes (7x) and the number of analysed drug concentrations or MPR (6x), respectively, in each analysis. The significance threshold was set to p≤0.001. Significant results are shown in red and nominal significant results are shown in bold.

		CD AMI (N=109)		CD VEN (N=256)		CD QUET (N=191)	CD MIR (N=169)	MPR NOR/AMI (N=61)	MPR ODM/VEN (N=129)	
	Diplotypes/Phenotypes	**P (Bonferroni)**	**Pairwise**	**P (Bonferroni)**	**Pairwise**	**P (Bonferroni)**	**P (Bonferroni)**	**P (Bonferroni)**	**P (Bonferroni)**	**Pairwise**
UGT2B7	*1/*1, *1/*2, *2/*2	0.609 (1)		0.850 (1)		0.746 (1)	0.893 (1)	0.487 (1)	0.853 (1)	
ABCB1	*1/*1, *1/*10, *1/*18, *1/*2, *1/*6, 2/*2, *2/*6, *2/*8, *2/*9, *2/*10, *2/*18, *6/*6, *6/*8	0.955 (1)		0.547 (1)		0.132 (1)	0.187 (1)	0.729 (1)	0.485 (1)	
CYP2C19	NM, IM, PM, RM, UM	0.994 (1)		**$5.67*10^{-5}$ (0.002)**		0.759 (1)	0.560 (1)	**0.009 (0.378)**	0.107 (1)	
	NM vs. IM				0.171					
	NM vs. PM				0.471					
	NM vs. RM				0.143					
	NM vs. UM				1.0					
	IM vs. PM				0.470					
	IM vs. RM				**$3.0*10^{-4}$**					
	IM vs. UM				**0.035**					
	PM vs. RM				0.202					
	PM vs. UM				0.131					
	RM vs. UM				1.0					
CYP2C9	NM, IM, PM	0.40 (1)		0.890 (1)		0.517 (1)	0.706 (1)	0.901 (1)	0.060 (1)	
ABCC2	*1/*1, *1/*2, *1/*UNK3, *2/*2, *2/*UNK3, *UNK2/*UNK3, *UNK3/*UNK3	0.149 (1)		0.996 (1)		0.678 (1)	0.770 (1)	0.329 (1)	0.224 (1)	
CYP1A2	*1A/*1A, *1A/*1F, *1A/*1L, *1F/*1F, *1F/*1L	0.386 (1)		0.372 (1)		0.333 (1)	0.646 (1)	0.596 (1)	**0.011 (0.462)**	

CYP2D6	NM, IM, PM; UM	**$7.90*10^{-4}$ (0.033)**	**0.003 (0.126)**	0.198 (1)	0.848 (1)	0.144 (1)	**$1.22*10^{-11}$ ($5.12*10^{-10}$)**
	NM vs. IM	0.103					**$3.3*10^{-8}$**
	NM vs. PM	**0.010**					**$2.7*10^{-5}$**
	NM vs. UM	0.500					0.442
	PM vs. IM	0.333					**$6.0*10^{-4}$**
	UM vs. IM	0.118					**0.028**
	UM vs. PM	0.079					0.096

CD, dose-corrected serum concentrations; MPR, metabolite-to-parent ratio; Ami, amitriptyline; Ven, venlafaxine; Quet, quetiapine; Mir, mirtazapine; Nor, nortriptyline; ODM, O-desmethylvenlafaxine; NM, normal metabolizer, IM intermediate metabolizer; PM, poor metabolizer; RM, rapid metabolizer; UM, ultrarapid metabolizer

Table 2 Diplotype/Phenotype analyses in regard to clinical improvement (percentual reduction in HAMD-21 score) were performed by Kruskal-Wallis tests. To adjust for alpha-error accumulation, nominal p-values were Bonferroni-corrected for the total number of genes (7x) and the number of analysed drug concentrations (4x) respectively, in each analysis. The significance threshold was set to p=0.002. Significant results are shown in red, nominal significant results are shown in bold.

		CI AMI (N=109)	CI VEN (N=256)	CI QUET (N=191)	CI MIR (N=169)
	Diplotypes/Phenotypes	**P (Bonferroni)**	**P (Bonferroni)**	**P (Bonferroni)**	**P (Bonferroni)**
UGT2B7	*1/*1, *1/*2, *2/*2	0.388 (1)	0.859 (1)	0.856 (1)	0.052 (1)
ABCB1	*1/*1, *1/*10, *1/*18, *1/*2, *1/*6, 2/*2, *2/*6, *2/*8, *2/*9, *2/*10, *2/*18, **6/*6, *6/*8	0.662 (1)	0.627 (1)	0.631 (1)	0.680 (1)
CYP2C19	NM, IM, PM, RM, UM	0.369 (1)	0.996 (1)	0.960 (1)	0.059 (1)
CYP2C9	NM, IM, PM	0.249 (1)	**0.011 (0.462)**	0.084 (1)	0.855 (1)
ABCC2	*1/*1, *1/*2, *1/*UNK3, *2/*2, *2/*UNK3, *UNK2/*UNK3, *UNK3/*UNK3	**0.020 (0.840)**	0.168 (1)	0.262 (1)	0.417 (1)
CYP1A2	*1A/*1A, *1A/*1F, *1A/*1L, *1F/*1F, *1F/*1L	0.353 (1)	0.415 (1)	0.265 (1)	0.194 (1)
CYP2D6	NM, IM, PM; UM	0.554 (1)	0.554 (1)	0.726 (1)	**0.037 (1)**

CI, clinical improvement; Ami, amitriptyline; Ven, venlafaxine; Quet, quetiapine; Mir, mirtazapine; Nor, nortriptyline; ODM, O-desmethylvenlafaxine;
NM, normal metabolizer, IM intermediate metabolizer; PM, poor metabolizer; RM, rapid metabolizer; UM, ultrarapid metabolizer

Table 3 Diplotype/Phenotype analyses in regard to remission were performed by chi-squared tests or Fisher´s exact tests. To adjust for alpha-error accumulation, nominal p-values were Bonferroni-corrected for the total number of genes (7x) and the number of analysed drug concentrations (4x) respectively, in each analysis. The significance threshold was set to p=0.002. Significant results are shown in red, nominal significant results are shown in bold.

		CI AMI (N=109)	CI VEN (N=256)	CI QUET (N=191)	CI MIR (N=169)
	Diplotypes/Phenotypes	**P (Bonferroni)**	**P (Bonferroni)**	**P (Bonferroni)**	**P (Bonferroni)**
UGT2B7	*1/*1, *1/*2, *2/*2	0.509 (1)	0.876 (1)	0.907 (1)	0.164 (1)
ABCB1	*1/*1, *1/*10, *1/*18, *1/*2, *1/*6, 2/*2, *2/*6, *2/*8, *2/*9, *2/*10, *2/*18, **6/*6, *6/*8	0.444 (1)	0.673 (1)	0.190 (1)	0.565 (1)
CYP2C19	NM, IM, PM, RM, UM	0.471 (1)	0.831 (1)	0.140(1)	0.249 (1)
CYP2C9	NM, IM, PM	1 (1)	0.473 (1)	0.731 (1)	0.897 (1)
ABCC2	*1/*1, *1/*2, *1/*UNK3, *2/*2, *2/*UNK3, *UNK2/*UNK3, *UNK3/*UNK3	**$3.4*10^{-4}$ (0.009)**	0.166 (1)	0.171 (1)	0.057 (1)
CYP1A2	*1A/*1A, *1A/*1F, *1A/*1L, *1F/*1F, *1F/*1L	0.742 (1)	0.945 (1)	0.657 (1)	0.654 (1)
CYP2D6	NM, IM, PM; UM	0.840 (1)	0.965 (1)	0452 (1)	0.678 (1)

CI, clinical improvement; Ami, amitriptyline; Ven, venlafaxine; Quet, quetiapine; Mir, mirtazapine; Nor, nortriptyline; ODM, O-desmethylvenlafaxine; NM, normal metabolizer, IM intermediate metabolizer; PM, poor metabolizer; RM, rapid metabolizer; UM, ultrarapid metabolizer

Supplement 5

to

Effects of Pharmacokinetic Gene Variation on Therapeutic Drug Levels and Antidepressant Treatment Response

Association between genotypes and haplotypes and serum concentrations and the combined effect of pk genes and serum concentrations on treatment response

Methods

Statistical analyses were conducted with PLINK v1.9 [1] and R v3.1.3 [2]. Single- and multi-marker associations were performed by logistic and linear regression models adjusted for sex and age. Pk analyses were conducted in PLINK with serum concentration as the outcome parameter. Interaction analyses were conducted in R with response data as outcome depending on the interaction of geno-/haplotypes and serum concentrations. Multi-marker interaction analyses were based on haplotypes showing the highest post probability for each individual after haplotype-phasing. To adjust for alpha-error accumulation, nominal p-values were Bonferroni-corrected for the total number of examined SNPs (32x) or haplotypes (33x) and the number of analysed drug concentrations or MPR (6x), respectively. The significance threshold for single marker analyses was set to $p \leq 2.6*10^{-4}$, and for haplotype analyses was set to $p \leq 2.5*10^{-4}$.

Computation of the statistical power was done with G*Power v3.1.9.2 [3]. In the two-tailed Wilcoxon signed-rank test, the sample reached a power of 86% for amitriptyline, 100% for venlafaxine, 97% for mirtazapine, and 98% for quetiapine treated patients to detect SNP and haplotype associations with an effect size of 0.3.

Results

Single marker analyses

Serum Concentrations

Out of 32 analyzed variants, only four markers located within the genes *CYP2C19* and *CYP2D6,* were significantly correlated with serum concentrations (Table 1).

Table 1 Significant results in single marker (SNPs) and haplotype analyses and where they are discussed (in main manuscript or supplement). To adjust for alpha-error accumulation, nominal p-values were Bonferroni-corrected for the total number of examined SNPs (32x) or haplotypes (33x) and the number of analysed drug concentrations or MPR (6x), respectively, in each analysis. The significance threshold was set for SNPs to $p \leq 2.6*10^{-4}$ and for haplotypes to $p \leq 2.5*10^{-4}$.

SERUM CONCENTRATION

GENE	Haplotype	SNP	m/M	Beta	P (Bonferroni)	Discussion
				CD Amitriptyline (N=109)		
CYP2D6		rs1135840	C/G	-0.37	$1.8*10^{-4}$ (0.035)	Supplement 5
		rs3892097	T/C	0.49	$1.7*10^{-5}$ (0.003)	Main manuscript
		rs1065852	A/G	0.50	$3.7*10^{-6}$ ($7.2*10^{-4}$)	Main manuscript
	*4			0.48	$2.4*10^{-5}$ (0.005)	Main manuscript
				CD Venlafaxine (N=256)		
CYP2C19		rs12248560	T/C	-0.34	$2.1*10^{-5}$ ($4.0*10^{-3}$)	Main manuscript
	*17			-0.35	$1.6*10^{-5}$ (0.003)	Main manuscript
				MPR O-desmethylvenlafaxine/Venlafaxine (N=129)		
CYP2D6		rs3892097	T/C	-2.14	$1.8*10^{-7}$ ($3.5*10^{-5}$)	Main manuscript
		rs1065852	A/G	-2.08	$2.3*10^{-7}$ ($4.5*10^{-5}$)	Main manuscript
	GCTCAAAAG			1.56	$6.7*10^{-5}$ (0.013)	Not discussed as this is no specific haplotype
	*4			-2.14	$1.8*10^{-7}$ ($3.6*10^{-5}$)	Main manuscript
				CD Mirtazapine (N=169)		
ABCB1	*8, *16			2.09	$5.7*10^{-5}$ (0.011)	Supplement 5

SNP, single nucleotide polymorphism; m/M, minor/Major allele; CD, dose-corrected serum concentrations; MPR, metabolite-to-parent ratio

Dose-corrected serum concentration of the active moiety (CD_{AM}) of amitriptyline was correlated with *CYP2D6* SNPs, such that in rs1135840 the minor (C) allele went along with significantly decreased CD_{AM} (p=0.035, ß=-0.37, $R2_{Adjusted}$=0.173), and in rs3892097 and rs1065852, both minor (T and A) alleles went along with significantly increased CD_{AM} (p=0.003, ß=0.49, $R2_{Adjusted}$=0.197; $p=7.2*10^{-4}$, ß=0.50, $R2_{Adjusted}$=0.220). Nominally significant associations of rs3892097 (p=0.019, ß=0.27, $R2_{Adjusted}$=0.054) and rs1065852 (p=0.037, ß=0.23, $R2_{Adjusted}$=0.033) with the MPR nortriptyline/amitriptyline did not withstand Bonferroni-correction.

The minor T-allele of the *CYP2C19* variant rs12248560 ($p=4.0*10^{-3}$, ß=-0.34; $R2_{Adjusted}$=0.183) showed lower CD_{AM} of venlafaxine than the major C-allele. In contrast the MPR O-desmethylvenlafaxine/venlafaxine was strongly associated with the two *CYP2D6* variants rs3892097 and rs1065852, both with the minor T- and A-alleles, respectively, conveying lower ratios ($p=3.5*10^{-5}$, ß=-2.14, $R2_{Adjusted}$=0.214; $p=4.5*10^{-5}$, ß=-2.08, $R2_{Adjusted}$=0.211).

For CD of mirtazapine and quetiapine, no association with any of the investigated SNPs could be detected.

Treatment Response

For amitriptyline, venlafaxine, mirtazapine, and quetiapine, neither an SNP nor the interaction between SNP and serum concentration was significantly associated with the response to drug therapy.

Haplotype analyses

Serum Concentrations

In line with single marker results, allele-specific differences with regards to CD_{AM} of amitriptyline and venlafaxine were found for haplotypes of *CYP2D6* and *CYP2C19* (Figure 1) additionally, one haplotype of *ABCB1* was associated with a CD of mirtazapine (Table 1).

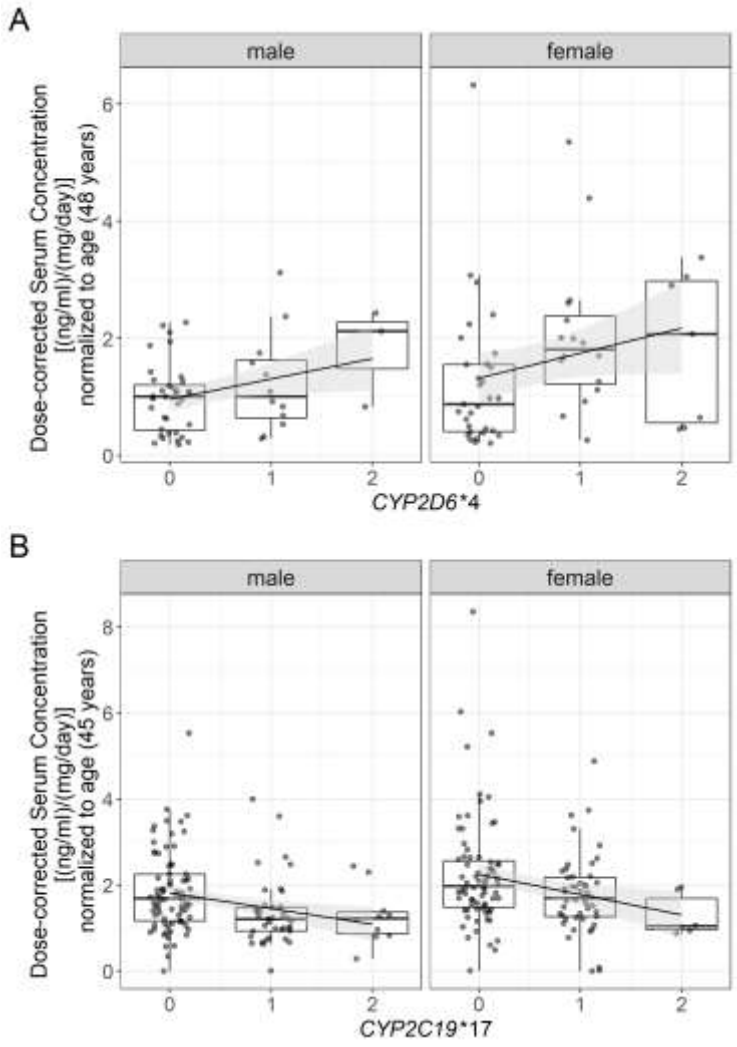

Figure 1 Dose-corrected serum concentrations normalised to the age of (A) amitriptyline were associated with *CYP2D64 and of (B) venlafaxine were associated with *CYP2C19**17 (linear regression analyses).** Linear regression analyses were corrected for sex and age; therefore dose-corrected serum concentrations were normalised to the mean age of each sample (48 and 45 years, respectively), and separate box-plot diagrams were prepared for male and female patients. Linear regression lines and the corresponding confidence intervals were

integrated into each diagram.

The *CYP2D6* haplotype *4, reflecting minor A- and T-allele associations of the included variants rs1065852 and rs3892097, was found to significantly impact CD_{AM} of amitriptyline. Carriers of this haplotype showed higher CD_{AM} (p=0.005, ß=0.48, $R2_{Adjusted}$=0.191) in contrast to carriers of other haplotypes (figure 1).

In accordance with results for the *CYP2C19* SNP rs12248560, the minor T-allele-containing haplotype *17 was significantly associated with lower CD_{AM} of venlafaxine (p=0.003, ß=-0.35, $R2_{Adjusted}$=0.185, figure 1). The *CYP2D6* haplotype *4 was associated with lower MPR ($p=3.6*10^{-5}$, ß=-2.14, $R2_{Adjusted}$=0.214). An additional *CYP2D6* haplotype GCTCAAAAG, comprising the minor A-allele of the nominally significant variant rs16947 (p=0.004, ß=1.13, $R2_{Adjusted}$=0.082), reached Bonferroni significance for association with increased O-desmethylvenlafaxine/venlafaxine ratio (p=0.013, ß=1.56, $R2_{Adjusted}$=0.137).

Higher CD of mirtazapine was significantly influenced by the *ABCB1* haplotype GAC (p=0.011, ß=2.09, $R2_{Adjusted}$=0.193).

CD of quetiapine again was not associated with the examined haplotypes.

Treatment Response

For amitriptyline, venlafaxine, quetiapine, and mirtazapine, none of the haplotypes nor the interactions between haplotypes and serum concentration showed a significant association with the response to drug therapy.

Discussion

In this section, only results that are not targeted in the main manuscript are discussed (Table 1).

We report, for the first time, that *CYP2D6* SNP rs1135840 was associated with amitriptyline serum concentration. This is a missense variant [4,5], which previously was associated with acute liver failure [5]. In our analysis, carriers of the minor C-allele showed lower serum concentrations compared to the wild type (G-allele). This is in accordance with previous studies, reporting an association of the minor allele with a higher rate of hydroxychloroquine metabolism [6] and an increased risk of adverse drug events in antituberculosis drug treatment in carriers of the GG-genotype [4]. As rs1135840 appears in different *CYP2D6* variants [7,8], it is not used to determine any particular variant.

Mirtazapine is mainly metabolized by CYP3A4, CYP1A2, and CYP2D6 [9]. The drug is not known as a substrate of p-glycoprotein [9,10]; however, in haplotype analyses, higher CD of mirtazapine was found in carriers of the *ABCB1* haplotype *8/*16 (GAC). P-glycoprotein

plays a role in limiting the bioavailability of drugs in the intestine, resulting in lower serum concentrations, but also in limiting the drug´s absorption into the brain [11,12]. Therefore, possibly this haplotype increased mirtazapine resorption due to a decrease in its efflux capacity. Single-marker analyses did not show an association of one of the included SNPs (rs1045642, rs1128503, rs2032582) with the CD of mirtazapine. The SNPs rs1128503 and rs1045642 are synonymous variants, and the triallelic SNP rs2032582 is a missense mutation, accounting for an altered amino acid function [11,13]. Literature on genetic associations of these SNPs with p-glycoprotein expression and function has largely been inconsistent; however, rs1045642 is most likely associated with altered protein expression and drug metabolism [11,13]. In line with single-marker analyses, haplotype analyses conducted previously were also inconsistent [12]. A prior analysis within the MARS sample investigating 95 SNPs within *ABCB1* showed that *ABCB1* affected antidepressant response in patients treated with p-glycoprotein substrates [14]. However, results addressing rs1045642, rs1128503, and rs2032582 were in accordance with our analyses, as these SNPs do not affect treatment response either [14]. Thus, the role of *ABCB1* in drug disposition and treatment response is still unclear [11].

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Supplement 6

to

Effects of Pharmacokinetic Gene Variation on Therapeutic Drug Levels and Antidepressant Treatment Response

Table 1 Diplotype/phenotype analyses in regard to serum concentrations were performed by Kruskal-Wallis tests. Patients receiving interacting drugs for CYP2D6 were excluded from the analysis. To adjust for alpha-error accumulation, nominal p-values were Bonferroni-corrected for the total number of genes (7x) and the number of analysed drug concentrations or MPR (6x), respectively, in each analysis. The significance threshold was set to p≤0.001. Significant results are shown in red and nominal significant results are shown in bold.

		CD AMI (N=109)		CD VEN (N=256)		CD QUET (N=191)	CD MIR (N=169)	MPR NOR/AMI (N=61)	MPR ODM/VEN (N=129)	
	Diplotypes/Phenotypes	**P (Bonferroni)**	**Pairwise**	**P (Bonferroni)**	**Pairwise**	**P (Bonferroni)**	**P (Bonferroni)**	**P (Bonferroni)**	**P (Bonferroni)**	**Pairwise**
CYP2D6	NM, IM, PM; UM	**0.001 (0.042)**		**0.009 (0.378)**		0.198 (1)	0.553 (1)	0.151 (1)	**$1.04*10^{-11}$ ($4.37*10^{-10}$)**	
	NM vs. IM		0.212							**$3.0*10^{-8}$**
	NM vs. PM		**0.009**							**$2.6*10^{-5}$**
	NM vs. UM		0.661							0.468
	PM vs. IM		0.166							**$2.9*10^{-4}$**
	UM vs. IM		0.189							**0.029**
	UM vs. PM		0.079							0.096

CD, dose-corrected serum concentrations; MPR, metabolite-to-parent ratio; Ami, amitriptyline; Ven, venlafaxine; Quet, quetiapine; Mir, mirtazapine; Nor, nortriptyline; ODM, O-desmethylvenlafaxine; NM, normal metabolizer, IM intermediate metabolizer; PM, poor metabolizer; RM, rapid metabolizer; UM, ultrarapid metabolizer

Table 2 Diplotype/phenotype analyses in regard to clinical improvement (percentual reduction in HAMD-21 score) were performed by Kruskal-Wallis tests. Patients receiving interacting drugs for CYP2D6 were excluded from the analysis. To adjust for alpha-error accumulation, nominal p-values were Bonferroni-corrected for the total number of genes (7x) and the number of analysed drug concentrations or MPR (6x), respectively, in each analysis. The significance threshold was set to p≤0.001. Significant results are shown in red and nominal significant results are shown in bold.

		CI AMI (N=109)	CI VEN (N=256)	CI QUET (N=191)	CI MIR (N=169)
	Diplotypes/Phenotypes	**P (Bonferroni)**	**P (Bonferroni)**	**P (Bonferroni)**	**P (Bonferroni)**
CYP2D6	NM, IM, PM; UM	0.600 (1)	0.932 (1)	0.557 (1)	**0.033 (1)**

CI, clinical improvement; Ami, amitriptyline; Ven, venlafaxine; Quet, quetiapine; Mir, mirtazapine; Nor, nortriptyline; ODM, O-desmethylvenlafaxine; NM, normal metabolizer, IM intermediate metabolizer; PM, poor metabolizer; RM, rapid metabolizer; UM, ultrarapid metabolizer