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

|                           | Combined Sample | Wuerzburg Sample | Munich Sample |
|---------------------------|-----------------|------------------|---------------|
| **Included patients**     | 109             | 62               | 47            |
| **Age [years]**           | 47.51 ± 12.60 (18-78) | 46.21 ± 12.96 (18-67) | 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** | 17.89 ± 10.06 (1-55) | 17.91 ± 10.60 (1-55) | 17.86 ± 9.55 (5-40) |
| **HAMD Baseline**         | 25.56 ± 6.61 (14-46) | 25.29 ± 7.22 (14-46) | 25.95 ± 5.67 (16-39) |
| **HAMD Out**              | 12.88 ± 7.08 (1-38) | 12.28 ± 6.79 (2-38) | 13.66 ± 7.44 (1-35) |
| **Length of disorder [years]** | 14.40 ± 12.43 (0-49) | 12.79 ± 11.71 (0-46) | 16.58 ± 13.16 (0-49) |
| **Dose (Out)**            | 142.8 ± 72.8 (25-340) | 115.7 ± 41.4 (25-225) | 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|                 |                  |               |
| Change of antidepressant due to adverse drug effects |                 |                  |               |
| Yes/No/Missing            |                 |                  |               |
| Type                      | Drowsiness/Sedation | Modified Salivation | Drowsiness/Sedation |
|                           | Modified Accommodation | Inner Restlessness |                   |
|                           | Weight gain       |                  |               |

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**                     |                          |                        |                      |
| 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            |                          |                        |                      |
| Type                      |                          | Drowsiness/Sedation    |                      |
|                           |                          | Modified Salivation    |                      |
|                           |                          | Inner Restlessness     |                      |
|                           |                          | Modified Accommodation |                      |
|                           |                          | Weight gain            |                      |
|                           |                          | Cardiovascular Effects |                      |
|                           |                          | Extrapyramidal Effects |                      |

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) |
| Included patients      | 171                     | 49.86 ± 14.22 (18-80)| 64                   | 48.58 ± 15.13 (18-80) |
| Age [years]            | 171                     | 49.86 ± 14.22 (18-80)| 64                   | 48.58 ± 15.13 (18-80) |
| 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) |
| 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   |                         | 62/1/1/0             |                      | 0/38/69             |
| NA-None/Mild/Medium/Severe |                  |                      |                      |                    |
| Change of antidepressant due to adverse drug effects | Yes/No/Missing 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 (0-49) | 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         |                  |

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

Adverse Drug Effects

| NA-None/Mild/Medium/Severe Change of antidepressant due to adverse drug effects Yes/No/Missing Type | Combined Sample | Wuerzburg Sample | Munich Sample |
|------------------------------------------------------------------------------------------------|-----------------|------------------|---------------|
|                                                                                                  | 99/5/1/0        |                  | 3/61/24       |

Drowsiness/Sedation Cardiovascular Effects Extrapyramidal Effects Drowsiness Other
Table presenting the combination of the SNPs according to the haplotypes.

| HAPLOTYPE | UGT2B7 | ABCB1 | CYP2C19 | CYP2C9 | ABC2 | CYP2D6 |
|-----------|--------|-------|---------|--------|------|--------|
|           | rs7662029 | rs7668258 | rs1045642 | rs1128503 | rs2032582 | rs12248560 | rs4244285 | rs17878459 | rs3758580 | rs1057910 | rs1799853 | rs2273697 | rs3740066 | rs717620 | rs2069514 | rs762551 | rs1065852 | rs28371725 | rs35742686 | rs3892097 | rs5030655 | rs5030656 | rs28371720 | rs16947 | rs1135840 |
|           | G       | C     | G    | G     | A    | C     | C       | A    | C     | T       | C     | C       | T       | T       | T       | A       | A       | A       | A       | A       | A       | G   | C     |
|           | A       | T     | G    | A     | G    | G     | G       | A    | A     | G      | G     | A       | T       | C       | C       | C       | C       | C       | C       | C       | C     |
|           | G       | G     | A    | A     | G    | A     | R       | G    | G     | R      | G     | G       | R       | G       | R       | G       | R       | G       | R       | R     |
|           | G       | C     | A    | C     | C    | A    | C       | A    | A     | T      | T     | C       | C       | C       | C       | C       | C       | C       | C     |
|           | A       | T     | C    | C     | A    | T     | C       | T    | C     | T      | T     | C       | T       | T       | T       | T       | T       | T     |
|           | G       | C     | G    | A     | A    | A     | A       | G    | G     | C      | C     | A       | A       | C       | G       | C       | C       | C     | C     |

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| 2; 21; | G | C | T | C | A | A | A | A | G |
| 28; e.g. | G | C | D | C | A | A | A | G | C |
| 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; | A | C | T | C | A | A | A | G | G |
| 37; e.g. | G | T | T | C | A | A | A | A | G |
| 32; 41 | G | T | T | C | A | A | A | A | G |
| 6 | G | C | T | C | D | A | A | G | C |
Supplement 3

to

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

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      |
| Trazodone          | 49 | 31     | Amisulpride        | 5  | 2      |
| Bupropion          | 49 | 21     | Haloperidol        | 4  |        |
| Paroxetine         | 46 | 24     | Clozapine          | 4  | 3      |
| Reboxetine         | 31 | 8      | Paliperidone       |    | 1      |
| Clomipramine       | 30 | 18     |                    |    |        |
| Doxepin            | 27 | 19     | Lamotrigine        | 144| 75     |
| Nortriptyline      | 16 | 14     | Pregabalin         | 45 | 15     |
| Fluoxetine         | 7  | 2      | Valproic Acid      | 27 | 16     |
| Imipramine         | 3  | 1      | Gabapentine        | 24 | 3      |
| Maprotiline        | 3  | 1      | Carbamazepine      | 12 | 5      |
| Milnacirpan        | 2  | 1      | Topiramate         | 5  |        |

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## Supplement 4

to

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

Table 1: Diploptotype/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 $p \leq 0.001$. Significant results are shown in red and nominal significant results are shown in bold.

| Diploptotypes/Phenotypes | 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) |
|--------------------------|----------------|----------------|----------------|----------------|---------------------|---------------------|
| **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/*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 | 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^{-11}) |
|--------|---------------------|---------------|-----------|-----------|-----------|-----------------------------|
| NM vs. IM | 0.103 | | | | | 3.3×10^{-4} |
| NM vs. PM | 0.010 | | | | | 2.7×10^{-4} |
| NM vs. UM | 0.500 | | | | | 0.442 |
| PM vs. IM | 0.333 | | | | | 6.0×10^{-4} |
| UM vs. IM | 0.115 | | | | | 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.

| Diplotypes/Phenotypes | CI AMI (N=109) | CI VEN (N=256) | CI QUET (N=191) | CI MIR (N=169) |
|-----------------------|----------------|----------------|-----------------|----------------|
| **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; 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.

| Gene       | Diplotypes/Phenotypes                  | P (Bonferroni) CI AMI (N=109) | P (Bonferroni) CI VEN (N=256) | P (Bonferroni) CI QUET (N=191) | P (Bonferroni) CI MIR (N=169) |
|------------|----------------------------------------|------------------------------|------------------------------|------------------------------|------------------------------|
| 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)                    | 0.045 (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
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 $p \leq 2.6 \times 10^{-4}$, and for haplotype analyses was set to $p \leq 2.5 \times 10^{-4}$.

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).
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 $p \leq 2.6 \times 10^{-4}$ and for haplotypes to $p \leq 2.5 \times 10^{-4}$.

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$_{AM}$ ($p=0.035$, $\beta=-0.37$, $R^2_{\text{Adjusted}}=0.173$), and in rs3892097 and rs1065852, both minor (T and A) alleles went along with significantly increased CD$_{AM}$ ($p=0.003$, $\beta=0.49$, $R^2_{\text{Adjusted}}=0.197$; $p=7.2 \times 10^{-4}$, $\beta=0.50$, $R^2_{\text{Adjusted}}=0.220$). Nominally significant associations of rs3892097 ($p=0.019$, $\beta=0.27$, $R^2_{\text{Adjusted}}=0.054$) and rs1065852 ($p=0.037$, $\beta=0.23$, $R^2_{\text{Adjusted}}=0.033$) with the MPR nortriptyline/amitriptyline did not withstand Bonferroni-correction.

The minor T-allele of the CYP2C19 variant rs12248560 ($p=4.0 \times 10^{-3}$, $\beta=-0.34$; $R^2_{\text{Adjusted}}=0.183$) showed lower CD$_{AM}$ of venlafaxine than the major C-allele. In contrast the MPR O-desmethylenvenlafaxine/venlafaxine was strongly associated with the two CYP2D6 variants rs3892097 and rs1065852, both with the minor T- and A-alleles, respectively, conveying lower ratios ($p=3.5 \times 10^{-5}$, $\beta=-2.14$, $R^2_{\text{Adjusted}}=0.214$; $p=4.5 \times 10^{-5}$, $\beta=-2.08$, $R^2_{\text{Adjusted}}=0.211$).

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](image.png)

**Figure 1** Dose-corrected serum concentrations normalised to the age of (A) amitriptyline were associated with $CYP2D6^*4$ 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$_{AM}$ ($p=0.005$, $\beta=0.48$, $R^2_{\text{Adjusted}}=0.191$) 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 ($p=0.003$, $\beta=-0.35$, $R^2_{\text{Adjusted}}=0.185$, figure 1). The **CYP2D6** haplotype *4 was associated with lower MPR ($p=3.6\times10^{-5}$, $\beta=-2.14$, $R^2_{\text{Adjusted}}=0.214$). An additional **CYP2D6** haplotype GCTCAAAAG, comprising the minor A-allele of the nominally significant variant rs16947 ($p=0.004$, $\beta=1.13$, $R^2_{\text{Adjusted}}=0.082$), reached Bonferroni significance for association with increased O-desmethylvenlafaxine/venlafaxine ratio ($p=0.013$, $\beta=1.56$, $R^2_{\text{Adjusted}}=0.137$).

Higher CD of mirtazapine was significantly influenced by the **ABCB1** haplotype GAC ($p=0.011$, $\beta=2.09$, $R^2_{\text{Adjusted}}=0.193$).

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.

| Diplotypes/Phenotypes | 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) |
|------------------------|---------------|----------------|-----------------|----------------|---------------------|----------------------|
|                        | P (Bonferroni) | Pairwise       | P (Bonferroni)  | Pairwise       | P (Bonferroni)      | P (Bonferroni)       |
| 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          |                 |                |                     |                      |
| NM vs. PM              |               | 0.009          |                 |                |                     |                      |
| NM vs. UM              |               | 0.661          |                 |                |                     |                      |
| PM vs. IM              |               | 0.166          |                 |                |                     |                      |
| UM vs. IM              |               | 0.189          |                 |                |                     |                      |
| UM vs. PM              |               | 0.079          |                 |                |                     |                      |

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 \(p\leq0.001\). Significant results are shown in red and nominal significant results are shown in bold.

| CYP2D6 | CI AMI (N=109) | CI VEN (N=256) | CI QUET (N=191) | CI MIR (N=169) |
|--------|---------------|----------------|-----------------|---------------|
| 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.