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Clinical Study

Polymorphisms in the Drug Transporter Gene *ABCB1* Predict Antidepressant Treatment Response in Depression

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SUMMARY

The clinical efficacy of a systemically administered drug acting on the central nervous system depends on its ability to pass the blood-brain barrier, which is regulated by transporter molecules such as *ABCB1* (MDR1). Here we report that polymorphisms in the *ABCB1* gene predict the response to antidepressant treatment in those depressed patients receiving drugs that have been identified as substrates of *ABCB1* using *abcb1ab* double-knockout mice. Our results indicate that the combined consideration of both the medication's capacity to act as an *ABCB1*-transporter substrate and the patient's *ABCB1* genotype are strong predictors for achieving a remission. This finding can be viewed as a further step into personalized antidepressant treatment.

INTRODUCTION

Antidepressants are the first-line treatment for major depression, but their overall clinical efficacy is unsatisfactory, as remission (i.e., full resolution of depressive symptoms) occurs in only one-third of the patients after a trial with an adequately dosed single drug (Trivedi et al., 2006), and remission rates further decline following successive treatment failures (Rush et al., 2006). This situation is particularly alarming in view of the fact that major depression constitutes one of the greatest disease burdens worldwide and is anticipated to be the second leading global disease burden by the year 2020, trailing only cardiovascular disease (Murray and Lopez, 1996). Apart from other causes, one possible reason for poor response to antidepressants is their inadequate penetration into the central nervous system, which depends on their ability to pass the blood-brain barrier (BBB). This barrier includes active transporters that are expressed at the luminal membrane of the endothelial cell lining, the small blood capillaries that form the BBB. These molecules actively transport their substrates against a concentration gradient out of the cells back into the blood circulation, thus potentially keeping brain drug concentrations low. One of the best-studied transporter molecules is P-glycoprotein (P-gp) (Cordon-Cardo et al., 1989; Thiebaut et al., 1987). P-gp is a member of the highly conserved superfamily of ATP-binding cassette (ABC) transporter proteins (Ambudkar et al., 1999). In humans, this 170 kDa glycoprotein is encoded on chromosome 7 by the *ABCB1* gene, also known as the multidrug resistance 1 (*MDR1*) gene (Callen et al., 1987). P-gp acts as an active efflux pump for a wide range of compounds, including a number of drugs and steroid hormones (Schinkel et al., 1996; Uhr et al., 2000, 2002). P-gp at the BBB thus regulates intracerebral concentrations and, by extension, may affect the clinical response of CNS-targeting drugs that are substrates of this transporter.

In this report we show that by using mice in which the homologs of the human *ABCB1* gene are deleted it is possible to classify which antidepressants are *ABCB1*-transporter substrates. The clinical usefulness of such a classification has been validated in a clinical study showing that *ABCB1* gene variants predict the treatment course and outcome in those patients treated with antidepressants that are *ABCB1*-transporter substrates.

RESULTS

Experiments Using *abcb1a* and *abcb1b* Double-Knockout Mice

Since P-gp function is only critical for drugs that are substrates of P-gp in humans, we developed an in vivo assay using mouse mutants lacking the homologs of the human *ABCB1* gene (i.e., *abcb1ab* knockout mice). After administration of antidepressants for 11 days with subcutaneously implanted osmotic infusion pumps, we observed that the intracerebral concentrations of citalopram ($F_{7,8} = 35.4$; $p < 0.001$), venlafaxine ($F_{7,8} = 30.7$; $p < 0.001$), and its active metabolite d-venlafaxine ($F_{7,8} = 4.5$; $p = 0.022$) are regulated by P-gp. The brain concentrations of citalopram, venlafaxine, and d-venlafaxine were 3.0, 1.7, and 4.1 times higher in the mutant mice compared to their wild-type littermates (see Table S1 available online). This was not the case for mirtazapine ($F_{8,9} = 3.0$; $p = 0.58$). No differences in plasma antidepressant concentrations were found between *abcb1ab* (−/−) mutants and their wild-type littermates for any of the drugs and metabolites investigated (Table S1).

The blood-organ barrier function is represented in Figure 1 as an organ/plasma concentration ratio. In the animals lacking P-gp, the penetration of citalopram, venlafaxine, and d-venlafaxine into the brain is 3.7, 1.8, and 3.6 times higher than in control

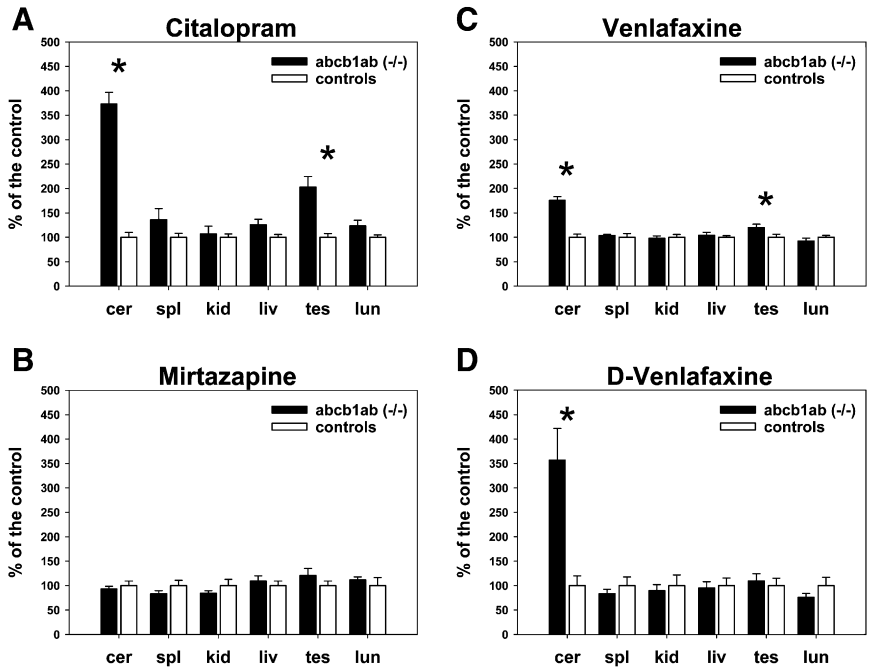

Figure 1. Blood-Organ Barrier Function for Antidepressant Drugs

Organ/plasma ratios of drug concentration in *abcb1ab*$^{-/-}$ mice compared to wild-type controls after subcutaneous administration of citalopram (A), mirtazapine (B), or venlafaxine (C and D) for 11 days via osmotic pumps. The organ/plasma ratios for citalopram (A), mirtazapine (B), venlafaxine (C), and desmethyl-venlafaxine (D) are shown as percentage of the control. An asterisk indicates a significant difference between the knockout mutants and the control mice (univariate F tests in MANOVA, p values < 0.05). Cerebrum (cer), spleen (spl), kidney (kid), liver (liv), testes (tes), and lung (lun) were investigated. Values are shown as means ± SEM.

animals. In addition, differences in the testis/plasma ratios were found for citalopram, venlafaxine, and d-venlafaxine in contrast to mirtazapine, which is in keeping with a blood-testis barrier regulated by P-gp.

Human SNP Association Study

If a patient is treated with a substrate of P-gp, functionally relevant genetic variants in the *ABCB1* transporter could influence intracerebral drug concentrations and thereby clinical response. To test this hypothesis, we analyzed the association of single-nucleotide polymorphisms (SNPs) in the *ABCB1* gene with the time until remission is achieved in patients under different antidepressants.

To that aim we investigated 95 SNPs in *ABCB1*, 74 of which were polymorphic. Information on chromosome position, putative function, HWE, minor allele frequencies (MAF), and the number of patients genotyped are presented in Table S2. The average intermarker distance of informative SNPs was 3.5 kb over the 262 kb long *ABCB1* region on chromosome 7, including all tagging SNPs of the HapMap project.

Because P-g does not influence cerebral penetration of all antidepressants, patients were divided in two subgroups: one group including patients treated with substrates of P-gp (citalopram, paroxetine, amitriptyline, and venlafaxine), the other one including patients treated with nonsubstrates (mirtazapine) (Uhr et al., 2000, 2003, 2007; Uhr and Grauer, 2003; Grauer and Uhr, 2004).

All polymorphic SNPs were tested for genotypic association with the phenotype remission after 4, 5, or 6 weeks.

Table S3 shows the empiric p values of this analysis determined by applying 10^6 permutations and corrected for age and sex for both the entire group and the two subgroups.

An association with p values of <0.001 was found only in the subgroup including patients who received drugs that were substrates of P-gp. The associated SNPs were rs2235067, rs4148740, rs10280101, rs7787082, rs2032583, rs4148739, rs11983225, rs10248420, rs2235040, rs12720067, and rs2235015.

This association analysis for all polymorphic SNPs and phenotypes was conducted according to Fisher's product method (FPM) (Fisher, 1932) as implemented in WG-Permer (http://www.wg-permer.org) for the genotypic and allelic models. Correction for multiple testing was performed using the minimum P method of Westfall and Young (1993). Table 1 shows the p values of FPM over all SNPs, sorted by phenotypes and models, as well as over all SNPs and all phenotypes, sorted by model and the Westfall-Young corrected p values. In the group of patients taking substrates of P-gp, there exist highly significant associations between the genetic variability of the SNPs tested and both the remission of depressive symptoms in weeks 4, 5, and 6 and the combination of these remission variables. In this group of patients, the Westfall and Young corrected p values over all SNPs and phenotypes was 0.00040 and 0.00016 for the genotypic and allelic analysis. In the group of patients taking drugs that are not substrates of P-gp, there was no significant association.

Figure 2 presents the genotype distribution of the SNP rs2032583 for patients who remitted after 4 weeks and those who did not. In the group of patients receiving antidepressants that are substrates of P-gp, there are clear differences in the genotype distribution between remitters and nonremitters (Figure 2A). While in the group of nonremitters patients carrying the C allele made up 9.5%, their percentage in the group of remitted patients was 45%. The 2 × 3 table Cochran-Armitage test yielded a χ^2 of 15.8, p = 0.00007, for patients receiving the P-gp substrates and a χ^2 of 0.004, p = 0.945, for patients receiving a non-P-gp substrate. If C carriers are treated with drugs that are substrates of P-gp, they have a markedly higher approximate relative risk for remission after 4 weeks. The odds ratio (approximate relative risk) was 7.72 (95% confidence interval limits 2.80 and 21.32) with p = 0.000065 in the 2 × 2 table Fisher's exact test. For any particular negative test result (noncarrier), the probability that it was true-negative (nonremission) was 81.5%.

Table 1. Fisher's Product and Westfall/Young Analysis

Fisher's Product and Westfall/Young Correction over All SNPs							
		All Patients		Substrates of P-gp		Nonsubstrates of P-gp	
Remission		Fisher's Product	Westfall/Young	Fisher's Product	Westfall/Young	Fisher's Product	Westfall/Young
4 weeks	genotypic	0.191	0.466	**0.00034**	**0.00011**	0.775	0.712
4 weeks	allelic	0.136	0.678	**0.00039**	**0.000048**	0.565	0.829
5 weeks	genotypic	**0.015**	0.132	**0.0084**	**0.0042**	**0.014**	0.056
5 weeks	allelic	**0.005**	**0.039**	**0.0019**	**0.0021**	0.160	0.137
6 weeks	genotypic	0.513	0.615	**0.0071**	**0.013**	0.644	0.621
6 weeks	allelic	0.327	0.233	**0.0012**	**0.00049**	0.904	0.856
Fisher's Product and Westfall/Young Correction over All SNPs and over All Phenotypes							
	genotypic	0.068	0.309	**0.00036**	**0.00040**	0.160	0.136
	allelic	**0.024**	0.099	**0.000094**	**0.00016**	0.557	0.340

An analysis of all polymorphic SNPs and phenotypes was conducted according to Fisher's product method (FPM) for the genotypic und allelic models. For FPM, as a single statistic is formed, no further correction for multiple testing is necessary. We also give result for the minimum P of Westfall and Young, which is a more conventional method controlling the family-wise error rate. Bold values indicate significant results.

When restricting the analysis to patients with unipolar depression (single episode and recurrent depression, n = 98), the odds ratio was 9.4 (95% confidence interval limits 3.0 and 29.4) with p = 0.000081.

For patients receiving non-P-gp substrates, there are no differences in the genotype distribution between remitters and nonremitters (Figure 2B). The odds ratio was 1.12 (95% confidence interval limits 0.38 and 3.37) with p = 1 in Fisher's exact test.

For a better presentation of the time course of remission up to week 6, a survival analysis (Cox regression) was performed. Figure 3 shows the decrease in nonremitters (i.e., patients who are still ill) for all patients and the two subgroups during the first 6 weeks depending on their genotype. For rs2032583, a distinction was made between C carriers and noncarriers; for rs 2235015 (an associated SNP located outside the linkage disequilibrium block containing rs2032583) between T carriers and noncarriers. The group of patients treated with a substrate of P-gp shows clear differences depending on the genotype. For instance, after 6 weeks, 62% of the patients who are non-C carriers of the SNP rs2032583 had not remitted, while this was the case in only 25% of the C carriers.

The values of the Wald statistics and p values for the other SNPs with highly significant association in the FPM are shown in Table S4 and confirm the association using FPM.

Case-Control Association

A control group of 362 subjects was genotyped for rs2032583 and rs2235015, the representative SNPs in the haplotype blocks 3 and 5 in the LD map (Figure S1). Subjects were randomly selected from a Munich-based community sample and negative for lifetime psychiatric axis I disorders (M-CIDI) and severe somatic diseases. Controls did not differ from the patient sample regarding gender distribution (p = 0.603), age (p = 0.322), or ethnicity (100% Caucasians in both samples). Genotype tests were performed using Fisher's exact test on a 2 × 3 table. We observed no significant case-control association for rs2032583 and rs2235015 (Fisher's exact test, p = 0.669 and 0.351).

DISCUSSION

To our knowledge, our results provide for the first time evidence that genetic variants in the *ABCB1* gene account for differences in the clinical efficacy of antidepressants, most likely by influencing their access to the brain. Here we report that antidepressant-induced remission of depressive symptoms is predicted by SNPs in the *ABCB1* gene among those depressed patients who were treated with drugs that are substrates of the *ABCB1*-encoded P-glycoprotein. To identify which antidepressants administered to patients are substrates of the P-glycoprotein,

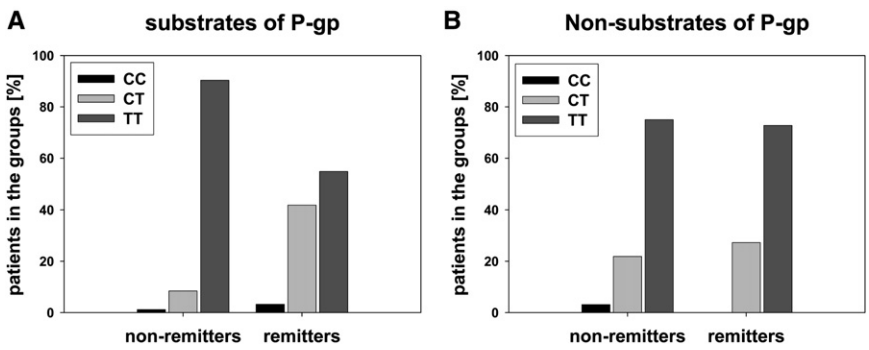

Figure 2. Distribution of rs2032583 Genotypes

Percentage of rs2032583 genotypes in the groups "nonremitters" and "remitters" for patients treated with substrates of P-gp (A) (amitriptyline, citalopram, paroxetine, or venlafaxine) and those treated with non-P-gp substrates (B) (mirtazapine).

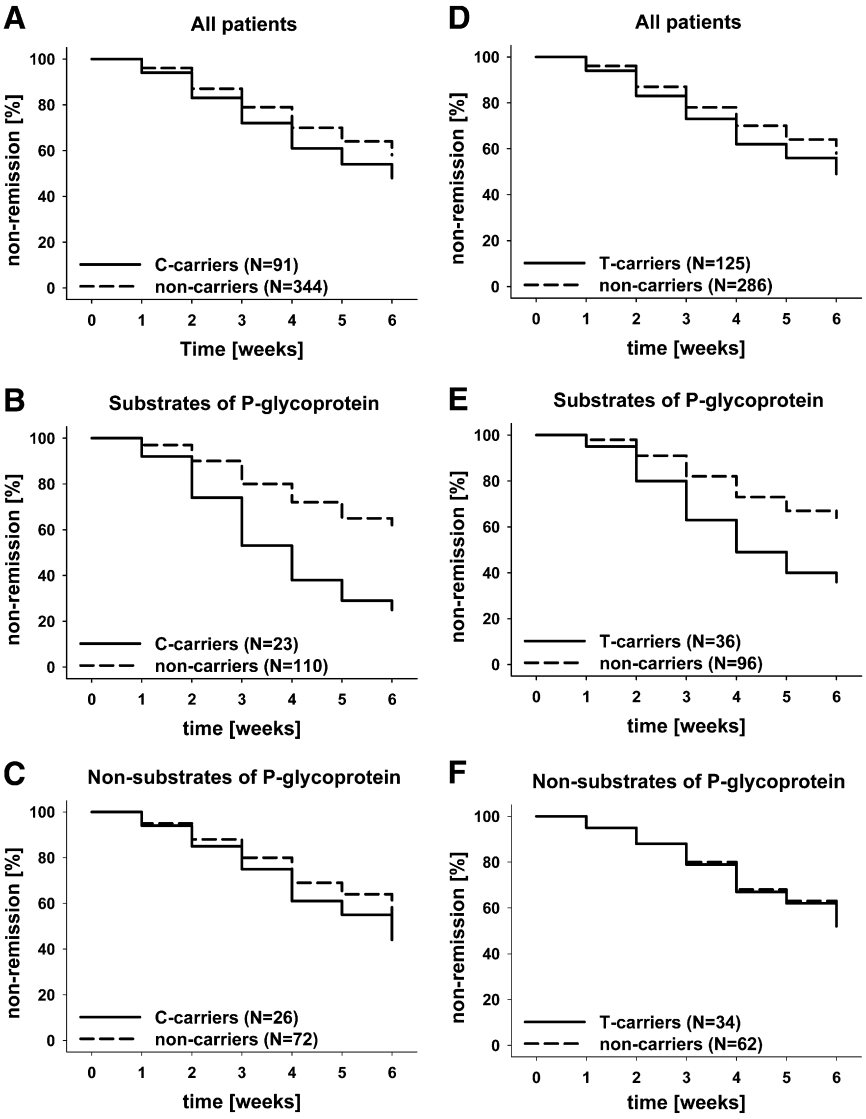

Figure 3. Cox Regression Analysis for rs2032583 and rs2235015

A Cox regression analysis for remission was performed for the SNPs 2032583 (A–C) and rs2235015 (D–F) dependent from the genetic feature "C carrier" (rs2032583) or "T carrier" (rs2235015). The Figure shows the time course of nonremitters (i.e., depressed patients). It depicts the examination of all patients (A and D) and the two subgroups of patients receiving substrates of P-gp (B and E) and those receiving non-P-gp substrates (C and F).

mouse mutants lacking the mouse homologs of the *ABCB1* gene were studied. Whereas P-gp is encoded by a single gene in humans (*ABCB1*), there are two homologs in mice, the *abcb1a* and *abcb1b* genes (Devault and Gros, 1990). Although *abcb1a* and *abcb1b* are not always expressed in the same organs, the overall distribution of these genes in mouse tissue coincides with that of the single *ABCB1* gene in humans, indicating that *abcb1a* and *abcb1b* together function in the same manner in the mouse as in human *ABCB1*. It has not yet been possible to predict the affinity of a substrate to P-gp from its chemical structure, hydrophobicity, lipophilicity, or charge. The mouse model reported herein allowed us to show in earlier studies that citalopram, venlafaxine, paroxetine, and amitriptyline were substrates of P-gp at the blood-brain barrier, while mirtazapine and fluoxetin were not (Uhr et al., 2000, 2003, 2007; Uhr and Grauer, 2003; Grauer and Uhr, 2004). This conclusion was drawn after administration of a single dose. In this study, the drugs were administered over an extended time period to investigate the time-dependent interaction between the drug and P-gp. We show that two structurally different antidepressants (citalopram and venlafaxine) were both substrates of P-gp at the BBB, as the brain drug concentration in mutants not carrying the genes coding for P-gp was increased (Table S1 and Figure 1). In contrast, the penetration of mirtazapine into the brain is not influenced by P-gp. The important role of P-gp was further supported by the fact that drug and metabolite organ concentrations and organ/plasma ratios of all antidepressants investigated did not differ between mutant and wild-type littermates in those organs that either lack P-gp completely or in which P-gp is not expressed in endothelial cells, such as the spleen, kidney, liver, and lung. These results show that some but not all antidepressants are substrates of P-gp in mice and, in addition, that this characteristic is seen after both single dose and repeated administration (Uhr et al., 2000, 2003; Uhr and Grauer, 2003).

To answer the question whether differences in the access of antidepressants into the brain might influence treatment outcome, we used the data from the Munich Antidepressant Response Signature (MARS) study (http://www.MARS-depression.de) in which we investigated the genetic variability of the *ABCB1* gene of 443 inpatients with depressive disorder receiving antidepressants.

Numerous papers describe polymorphisms in *ABCB1* (Hoffmeyer et al., 2000; Kim et al., 2001; Ito et al., 2001; Cascorbi et al., 2001; Tanabe et al., 2001; Cascorbi, 2006), and a multitude of single-nucleotide polymorphisms (SNPs) are listed in public SNP databases. The genetic variability of all polymorphic SNPs studied and the recovery from depressive symptoms were examined in a summarizing model using Fisher's product method. After correction for multiple testing, we found a highly significant association between the genotypes or allele frequencies of the *ABCB1* gene and remission in weeks 4, 5, and 6 only in patients receiving substrates of P-gp (Table 1). For a better presentation

of the clinical course and involvement of the entire period up to week 6 including all patients enrolled in the study, a Cox regression survival analysis was performed. As demonstrated in Figure 3 and Table S4, only those patients receiving substrates of P-gp showed genotype-dependent, highly significant target parameter differences during the clinical course, achieving a remission with a score of <10 on the HAM-D-scale.

As exemplified in Figure 2 for the SNP rs2232583, the genotype distribution differs between the patient group with remission versus the group without remission among patients treated with a P-gp substrate for 4 weeks. The probability of being remitted from depression after 4 weeks is increased for C carriers treated with substrates of P-gp (odds ratio: 7.72, p = 0.000065). In contrast, it was predictable that patients carrying the TT genotype will not be remitted after a 4 week treatment period. In the group of patients receiving the nonsubstrate mirtazapine, there was no difference found between the genotypes of remitters and nonremitters. The group characteristics for the representative SNP rs2032583, depending on the genotype, did not differ between the two subgroups "patients treated with P-gp substrates" and "patients treated with non-P-gp substrates" (Table S5).

The Cox regression analysis did not reveal any significant differences for remission rates (HAM-D < 10) in the first 6 weeks (Wald value = 0.125; sig = 0.72) between the subgroup of patients receiving drugs that are substrates of P-pg and the subgroup of patients receiving drugs that are not substrates of P-gp, allowing us to reject the possibility that differences in drug efficacy account for our different findings. In fact, the drugs used here proved to be especially effective according to large comparative studies required for approval. It is only the combined consideration of both the patient's *ABCB1* genotype and the medication's P-gp substrate status that identifies a group of patients exhibiting a clearly better remission rate than patient populations assembled on the basis of the patient's genotype or the medication's P-gp substrate status alone.

SNPs in *ABCB1* have been reported to influence intestinal uptake and thus plasma levels of drugs (Hoffmeyer et al., 2000; Sakaeda et al., 2003). However, the various genotype-dependent remission rates found in this study were not due to different doses or plasma drug levels, which confirms that monitoring plasma antidepressant levels is not reliable to predict adequacy of treatment (Supplemental Data).

All highly associated SNPs are located in introns and with the exception of rs2235015 located in a single haplotype block (Figure S1). The SNPs rs2235067, rs4148740, rs2032583, rs4148739, rs11983225, s2235040, and rs12720067, as well as the SNPs rs7787082 and rs10248420, exhibit an r^2 of >0.8 with each other. This high degree of LD proscribes to single one of them out as a potential functional variant based on the strength of association only. We speculate that SNPs in LDs with these or a haplotype thereof influence intronic regulatory elements located between exons 10 and 24. In fact, resequencing all exons and exon-intron junctions excluded the possibility that the potential functional polymorphism could be exonic or alter splicing (Supplemental Data). In addition, the LD block containing all associated SNPs in our studies as well as the ones known in HapMap is separated from all predicted promoter elements as well as the neighboring genes by recombination hotspots (Figures S1 and S2), making it unlikely that the associated SNPs are markers for functional variants in these areas. Furthermore, the area delimited by these SNPs contains several highly conserved intronic regions as well as two regions with a high density of human-mouse-rat conserved transcription factor binding sites (Figure S3). This could be related to the presence of evolutionarily conserved functional intronic regulatory regions, which are gaining more and more importance (The ENCODE Project Consortium, 2007).

Recently, McMahon et al. (2006) reported that African-Americans responded less well to an established antidepressant than Caucasians. They attributed this difference to the higher frequency of the A allele in a specific SNP in the serotonin 2 A receptor gene. In this context, it is to be noted that the *ABCB1* gene and in particular the SNPs described by us predicting the therapeutic outcome exhibit considerable ethnic differences (Kim, 2002; Tang et al., 2002, 2004; Kroetz et al., 2003). Thus, the current finding indicates that variant *ABCB1* genotypes contribute to differences in treatment outcome across ethnic groups and further encourages studies to elucidate the clinical implications of these differences across ethnic groups.

The general conclusion to be drawn is that any drug administered to treat CNS diseases should be analyzed for its P-gp substrate status, which can be determined by using *abcb1ab* knockout mice. From a clinical point of view, the findings warrant that patients receiving a drug that is a P-gp substrate for the treatment of brain diseases are genotyped to exclude the possibility that a patient receives a drug that fails to enter the CNS to an extent required for efficacy. The combination of therapeutic drug monitoring (TDM) involving enteral drug intake and cytochrome P450 drug metabolism and P-gp genotyping for detecting the drug's bioavailability in the brain may predict the response of an individual patient to a certain drug. In addition, side effects of drugs acting on the CNS may be caused by a weakened P-gp function. Thus, knowing the P-gp status could make it possible to predict central side effects of drugs both acting on the CNS and peripherally.

Finally, the interdependence of P-gp substrate capacity and *ABCB1* genotype needs to be considered in future drug development, because drugs that differ in their P-gp substrate capacity need to be evaluated in clinical trials where study populations are stratified according to the *ABCB1* genotype.

EXPERIMENTAL PROCEDURES

Experiments Using Transgenic Animals

Materials and Animals

Materials and animals were already described (Uhr et al., 2003; Uhr and Grauer, 2003) and are represented in the Supplemental Data section. All animal experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals of the Government of Bavaria, Germany. Age, weight, and group size of the mice used are shown in Table S8.

Experimental, Extraction Procedures, HPLC, and Statistics

Citalopram, mirtazapine, and venlafaxine dissolved in 0.9% sodium chloride and 0.5% ethanol were administered subcutaneously in the nape of the neck through surgically implanted osmotic infusion pumps (Alzet micro-osmotic pump, Alza Corporation, Palo Alto, CA, USA), which continuously delivered the drugs at the scheduled concentrations (citalopram and mirtazapine 60 μg/d; venlafaxine 300 μg/d). After 11 days, the mice were anesthetized and sacrificed. The dissected organs were homogenized, a liquid-liquid extraction

procedure was performed, and high-performance liquid chromatography (HPLC) measurements and statistics were carried out as described elsewhere (Uhr et al., 2003, and Supplemental Data).

Human Genetic Studies

Description of Patients

The study included 443 inpatients with depression who were treated at the Max Planck Institute of Psychiatry (MPI), Munich/Germany.

Patients were included in this study within 1–3 days of admission and diagnosed by trained psychiatrists according to the Diagnostic and Statistical Manual of Mental Disorders (DSM) IV criteria. Patients with depressive disorder due to a medical or neurological condition were excluded. Ethnicity was recorded using a self-report sheet for nationality, native language, and ethnicity of the subject and of all four grandparents. The study was approved by the local ethics committee and written informed consent obtained from all subjects.

The study was designed as a pharmacogenetic study (Munich Antidepressant Response Signature [MARS] project) (Binder et al., 2004) to discover biomarkers and genotypes predictive of clinical outcome in relationship to treatment, which was at doctor's choice. The antidepressant plasma concentrations were monitored to ascertain clinically efficient drug levels.

Patients were subdivided into two groups according to the antidepressant property as P-gp substrate. Patients taking antidepressants that are substrates of P-gp received amitriptyline, paroxetine, venlafaxine, or citalopram, and patients taking antidepressants that are not substrates of P-gp received mirtazapine for at least 4 weeks within the first 5 weeks of treatment. In addition, they were not allowed to take other antidepressants for more than 3 weeks (with the exception of trimipramine, which was allowed in a daily dose of up to 100 mg to facilitate sleep). If a patient was discharged prior to week 5, she/he had to take the respective drugs for 3 weeks when hospitalized for 4 weeks and for 2 weeks in the case of a 3 week hospitalization. The group characteristics number of patients (% women), age, Hamilton Depression Rating Scale (HAM-D) at inclusion, age at onset, illness duration in years, number of previous episodes, number of former hospitalizations, duration of actual episode, ethnicity, comedication, and diagnosis are shown in Tables S5 and S6 for the representative SNPs rs2032583 and rs2235015 depending on the genotype.

Not all patients finished the weekly psychopathology ratings. After weeks 4, 5, and 6, the data of only 366 (83%), 324 (73%), and 297 (67%) patients were available for evaluation. This attrition was due to the patients' rapid recovery, discharge against doctor's advice, or refusal of further participation. After discharge from the hospital, no further weekly psychopathology ratings were collected.

Psychopathology and Definition of Response to Antidepressant Drug Treatment

Trained raters using the 21 item HAM-D scale assessed the severity of psychopathology at admission. Patients fulfilling the criteria for at least one moderate depressive episode (HAM-D $\geq$ 14) entered the analysis. Ratings were performed within 3 days of admission and then weekly until discharge.

Remission was defined as reaching a total HAM-D score of less than 10. The time period was chosen because it is considered to be necessary for an antidepressant drug to display its clinical efficacy.

DNA Preparation, SNP Selection, and Genotyping

Forty milliliters of EDTA blood was drawn from each patient, and DNA was extracted using the Puregene whole-blood DNA extraction kit (Gentra Systems, Minneapolis, MN, USA).

Ninety-five *ABCB1* (NM_000927) SNPs were genotyped. SNPs were selected from dbSNP (http://www.ncbi.nlm.nih.gov:80/) or part of the Illumina Sentrix Human-1 100 k BeadChips.

Genotyping was performed on a matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometer (MassArray system) employing the Spectrodesigner software (Sequenom; San Diego, CA, USA) for primer selection and multiplexing and the homogeneous mass extension (hME) process for producing primer extension products. Cleaned extension products were analyzed by means of a mass spectrometer (Bruker Daltronik) and the peaks identified using the SpectroTYPER software (Sequenom). Only if the call rate of a plate was higher than 90% were the genotypes used for evaluation.

SNPs that could not be examined by means of the Sequenom technology were tested by pyrosequencing (Biotage, Uppsala, Sweden).

rs7779562, rs4148738, rs2235033, rs10264856, rs10267099, rs7796247, and rs10275625 were performed on Sentrix Human-1 Genotyping BeadChips (Illumina Inc., San Diego, CA, USA) according to the manufacturer's standard protocols. The SNPs rs2235048, rs1045642, rs2032583, rs2235046, rs1202169, rs2235015, and rs1202172 were performed using both the Sequenom and Illumina technology. The results were congruent in more than 99%.

The tetra-allelic SNP rs2032582 was measured with the light-cycler using allele-specific hybridization probes.

Statistics

Exact tests to detect deviations from Hardy Weinberg equilibrium (HWE) (Wigginton et al., 2005) were performed for all SNPs (Table S2).

To calculate whether there exists a significant association between phenotype variables and genetic variability in the *ABCB1* gene, the multivariate Fisher's product method (FPM) was employed. Phenotype variables were corrected for the effects of age and gender by calculating linear regression residuals using the statistic package software SPSS Release 14.0.0 (Chicago, IL, USA). Bivariate associations between SNP genotyping data and phenotypes were tested in an ANOVA analysis. Empiric p values were calculated by applying 10^6 permutations (phenotypes randomly redistributed over the genotypes). Fisher's product method (FPM) (Fisher, 1932) includes the residuals of the phenotype variables remission after 4 weeks, remission after 5 weeks, remission after 6 weeks, and the genotype variables from all polymorphic SNPs. P value correction for multiple comparisons was done by resampling (10^6 permutations).

To correct for multiple testing, the permutation method by Westfall and Young (1993) was applied to take advantage of the dependence structure between the SNPs and the phenotypes. We performed the entire analysis scan 10^6 times using phenotypes randomly redistributed over the genotypes.

Finally, a Cox regression survival analysis was carried out with the statistic package software SPSS Release 14.0.0 (Chicago, IL, USA), examining remission incidence (HAM-D < 10) during the first 6 weeks of treatment, with age and gender as covariates.

Supplemental Data

The Supplemental Data for this article can be found online at http://www.neuron.org/cgi/content/full/57/2/203/DC1/.

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