ORIGINAL ARTICLE

A Genomewide Association Study Points to Multiple Loci That Predict Antidepressant Drug Treatment Outcome in Depression

Marcus Ising, PhD; Susanne Lucae, MD, PhD*; Elisabeth B. Binder, MD, PhD; Thomas Bettecken, MD; Manfred Uhr, MD, PhD; Stephan Ripke, MD; Martin A. Kohli, MSc; Johannes M. Hennings, MD; Sonja Horstmann, MD; Stefan Kloiber, MD; Andreas Menke, MD; Brigitta Bondy, MD; Rainer Rupprecht, MD; Katharina Domschke, MD, MA; Bernhard T. Baune, MD, PhD, MPH; Volker Arolt, MD; A. John Rush, MD; Florian Holsboer, MD, PhD; Bertram Müller-Myhsok, MD*

Context: The efficacy of antidepressant drug treatment in depression is unsatisfactory; 1 in 3 patients does not fully recover even after several treatment trials. Genetic factors and clinical characteristics contribute to the failure of a favorable treatment outcome.

Objective: To identify genetic and clinical determinants of antidepressant drug treatment outcome in depression.

Design: Genomewide pharmacogenetic association study with 2 independent replication samples.

Setting: We performed a genomewide association study in patients from the Munich Antidepressant Response Signature (MARS) project and in pooled DNA from an independent German replication sample. A set of 328 single-nucleotide polymorphisms highly related to outcome in both genomewide association studies was genotyped in a sample of the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) study.

Participants: A total of 339 inpatients with a depressive episode (MARS sample), a further 361 inpatients with depression (German replication sample), and 832 outpatients with major depression (STAR*D sample).

Main Outcome Measures: We generated a multilocus genetic variable that described the individual number of alleles of the selected single nucleotide polymorphisms associated with beneficial treatment outcome in the MARS sample ("response" alleles) to evaluate additive genetic effects on antidepressant drug treatment outcome.

Results: Multilocus analysis revealed a significant contribution of a binary variable that categorized patients as carriers of a high vs low number of response alleles in the prediction of antidepressant drug treatment outcome in both samples (MARS and STAR*D). In addition, we observed that patients with a comorbid anxiety disorder combined with a low number of response alleles showed the least favorable outcome.

Conclusion: These results demonstrate the importance of multiple genetic factors combined with clinical features in the prediction of antidepressant drug treatment outcome, which underscores the multifactorial nature of this trait.

Arch Gen Psychiatry. 2009;66(9):966-975

ANTIDEPRESSANT AGENTS ARE indispensable in treating severe depression. Since their discovery in the 1950s, adverse effect profiles of these drugs have been improved, whereas clinical efficacy is still unsatisfactory because 1 in 3 patients does not fully recover from depression, even after several treatment trials.[1,2] Genetic factors contribute to the general variability in drug response[3,4] and, according to family studies,[5-7] this is the case for antidepressant agents, which suggests that the individual genetic profile may provide guidance in medication selection.[8] Up to now, pharmacogenetic studies have focused on candidate genes implicated in the mechanisms of antidepressant drug action or in the pharmacokinetics of such drugs. For example, an insertion-deletion polymorphism in the promoter region of the serotonin transporter gene (*SLC6A4*) seems to predict response to selective serotonin reuptake inhibitors,[9] potentially mediated by differences in selective serotonin reuptake inhibitor tolerability,[10] and a variation in the *ABCB1* gene coding for a P-glycoprotein that determines brain tissue penetration of many antidepressant drugs may predict clinical outcome in patients treated with substrates of this blood-brain barrier regulation molecule.[11] Several studies reported that variants of a gene coding for *FKBP5*,[12-14] a co-chaperone involved in stress hormone signaling, and variants for *5HT2A*[15] are predictive of treatment response but do not effectively guide

Author Affiliations are listed at the end of this article.
*Drs Ising and Lucae contributed equally to this article; their names are listed in alphabetical order.

©2009 American Medical Association. All rights reserved.
Downloaded From: http://jamanetwork.com/ on 05/19/2013

Table 1. Demographic and Clinical Sample Characteristics

	MARS Sample (n=339)	German Replication Sample (n=361)	STAR*D Sample (n=832)	*P* Value[a]
Women, %	56.0	60.7	57.9	.46
Age, mean (SD), y	49.0 (14.5)	48.8 (14.4)	42.9 (13.5)	<.001
White, %	100	100	100	>.99
Depression diagnoses, No. (%)				<.001
Single depression episode	90 (26.5)	92 (25.5)	178 (21.4)	
Recurrent depression	211 (62.2)	213 (59.0)	654 (78.6)	
Bipolar disorder	38 (11.2)	56 (15.5)	0	
Comorbid anxiety, No. (%)	24 (7.1)	104 (28.8)	155 (18.6)	<.001
Illness-related variables, mean (SD)				
Age at onset, y	37.5 (15.7)	37.1 (13.6)	25.8 (14.6)	<.001
Duration of current episode, wk	40.5 (73.1)	NA	94.6 (218)	<.001
QIDS-C score at inclusion[b]	18.4 (4.2)	17.0 (4.6)	15.9 (3.1)	<.001

Abbreviations: MARS, Munich Antidepressant Response Signature; NA, not available; QIDS-C, clinician rating version of the Quick Inventory of Depressive Symptomatology; STAR*D, Sequenced Treatment Alternatives to Relieve Depression.

[a] *P* values (2-tailed) of Pearson χ^2 tests (qualitative data) and univariate analysis of variance (quantitative data) are reported.

[b] Hamilton Depression Rating Scale values from the MARS and the German Replication samples were translated into QIDS-C scores according to the conversion table suggested by Rush et al.[21]

treatment selection. Further associations have been reported for the glutamatergic receptor gene *GRIK4*,[16] the enzymatic gene *PDER11A*,[17] inflammation-related genes (*CD3E, PRKCH, PSMD9,* and *STAT3*),[18] and *UCN3*[18] expressing a ligand of the CRF2 receptor.

Because the mechanisms by which antidepressant agents exert their clinical effects are not yet fully understood, studies that focus on single candidate genes may not identify novel genetic information of clinical importance. Therefore, we conducted an unbiased genomewide pharmacogenetic study in patients undergoing antidepressant drug treatment to discover new gene variants that contribute to a favorable outcome. Furthermore, treatment response is not only determined by genetic makeup but also by course of illness, comorbid anxiety, age at disease onset, current age, and sex.[1,19,20] These variables were additionally considered to determine whether they predict the outcome of antidepressant drug treatment.

METHODS

We report the results of 2 genomewide association studies (GWASs). In the first study, we genotyped patients from the Munich Antidepressant Response Signature (MARS) project[1]; in the second study, we determined genomewide allele frequencies in pooled DNA from a German replication sample. Subsequently, a set of single-nucleotide polymorphisms (SNPs) highly related to outcome in both GWASs was genotyped in a sample from the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) study,[2] a multicenter treatment trial that uses a series of standard treatments in an outpatient sample. We were encouraged to use the MARS project and the STAR*D study as discovery and replication samples, respectively, because several concordant pharmacogenetic findings in candidate gene studies emerged from both.[12,14,15]

MARS SAMPLE

A total of 339 inpatients from the MARS project[1] with major depression (88.8%) or bipolar disorder (11.2%) were included within 1 to 5 days of admission as inpatients (**Table 1**). Diagnosis was ascertained by trained psychiatrists according to the *DSM-IV*[22] criteria. The exclusion criteria were the presence of alcohol or substance abuse or dependence (including eating disorders with concomitant laxative abuse), comorbid somatization disorder, and depressive disorders owing to general medical or neurologic conditions. Ethnicity was recorded by the use of a self-report questionnaire that asked for the nationality, first language, and ethnicity of the participant and all 4 grandparents. All the patients were white, and 85.1% were of German descent; the remaining patients were of European descent (central Europe, 6.5%; eastern Europe, 7.8%; and Mediterranean, 0.6%). The study was approved by the local ethics committee of the Ludwig Maximilians University of Munich, and written informed consent was obtained from all the participants.

The severity of the psychopathologic abnormality was assessed by trained raters by the use of the 21-item Hamilton Depression Rating Scale (HAM-D).[23] Patients with at least moderately severe depression (HAM-D score ≥14) entered the analysis. Ratings were performed within 3 days of hospital admission and then weekly until discharge. We used 3 common types of response definitions, each of which defined different aspects of antidepressant drug treatment outcome: early partial response (HAM-D score reduction ≥25% after 2 weeks of treatment), response (HAM-D score reduction ≥50% after 5 weeks of treatment), and remission (HAM-D score <10, evaluated after 5 weeks and before discharge from the hospital). The MARS project was designed as a naturalistic pharmacogenetic study in which all patients were treated with antidepressant agents according to the choice of the physicians; plasma antidepressant drug concentrations were monitored to ensure clinically efficient drug levels.

GERMAN INPATIENT REPLICATION SAMPLE

The German replication sample consisted of 361 inpatients from the psychiatric hospitals of Ludwig Maximilians University and the University of Muenster (Table 1). Sex distribution ($P > .2$) and age ($P > .9$) did not differ between samples. Overall, 84.5% of these patients had major depression, whereas 15.5% were in a depressive episode of a bipolar disorder. Trained psychiatrists ascertained *DSM-IV* diagnosis. Patients were rated weekly from hospital admission to discharge (Munich) or until week 6 (Muenster) by the use of the 21-item HAM-D. Ethnicity was recorded by the use of the same self-report questionnaire as in the MARS project. All the patients were white, and 90.7% were

©2009 American Medical Association. All rights reserved.
Downloaded From: http://jamanetwork.com/ on 05/19/2013

of German descent; the remaining patients were of European descent (central Europe, 3.9%; eastern Europe, 5.3%; and Mediterranean, 0.1%). The same inclusion and exclusion criteria applied as in the MARS sample, and outcome with antidepressant drug treatment was evaluated accordingly.

STAR*D REPLICATION SAMPLE

A subsample of 832 outpatients from the STAR*D study[20,24] was selected as a second replication sample. The selection criteria were white ethnicity, a score of at least 10 on the 16-item clinician rating version of the Quick Inventory of Depressive Symptomatology (QIDS-C)[21] at study inclusion (corresponding to a HAM-D score ≥14), and availability of QIDS-C data for at least the first 2 weeks of treatment. In agreement with the selection criteria of the MARS project and the German replication sample, patients with a concurrent alcohol or substance use disorder, bulimia, or somatization disorder diagnosed using the Psychiatric Diagnostic Screening Questionnaire[25] were excluded. In addition, 12 individuals were excluded owing to low genotyping quality. All the patients participated in the first treatment step (level 1) of the STAR*D study and received citalopram hydrobromide. To identify partial response, response, and remission in a manner consistent with the two German studies, we selected QIDS-C scores that corresponded to the HAM-D scores used in the initial samples following published conversion recommendations.[21] For demographic and clinical characteristics of this STAR*D sample, see Table 1.

CONTROL SUBJECTS FOR THE CASE-CONTROL ANALYSIS

A total of 366 control individuals (161 men and 205 women; mean [SD] age, 48.6 [13.4] years) who were matched to the MARS sample for ethnicity (using the same questionnaire), sex, and age were recruited at the Max Planck Institute of Psychiatry. They were selected randomly from a Munich-based community sample. The exclusion criteria were the presence of severe somatic diseases and a lifetime history of Axis I mental disorders. The latter was ascertained using the Munich version of the Composite International Diagnostic Interview.[26]

SNP GENOTYPING

Genotyping in the MARS sample was performed by the use of 2 types of whole-genome genotyping arrays: Sentrix Human-1 (109 000 loci) and HumanHap300 (317 000 loci) BeadChip (Illumina Inc, San Diego, California), which together covered almost 410 000 nonoverlapping SNPs from the entire human genome. Genotyping was performed according to the standard protocols of the manufacturer. We excluded SNPs with a call rate of less than 98%, with a deviation from Hardy-Weinberg equilibrium (HWE) at an error level of less than 10^{-5}, or with a minor allele frequency (MAF) less than 2.5%, which resulted in 93 339 SNPs from the Sentrix Human-1 chip and 295 912 SNPs from the HumanHap300 chip. A total of 4.5% of all analyzed SNPs showed a nominally significant deviation from HWE with the level of significance set to 5%, which is almost identical to the expected number of false-positive findings under the null hypothesis of missing HWE deviations (P=.83). The average casewise call rate across all SNPs was 99.8%, and the reproducibility for samples (n=3) genotyped twice was 99.999%. A test for population stratification with 10 000 random SNPs as genomic controls showed no evidence of admixture.

Genomewide allele frequencies in the German replication sample were determined separately in 3 pairs of pools that contained the DNA of patients with (1) early partial response vs nonresponse, (2) response after 5 weeks vs nonresponse, and (3) remission after 5 weeks vs nonremission. The DNA of responders after 5 weeks was pipetted in duplicate for quality control. Owing to technical restrictions, genomewide analysis of the pooled DNA samples was performed by the use of only the HumanHap300 (317 000 loci) BeadChip. All the pools were measured 3 times, except the duplicated DNA pools of 5-week responders, which were measured twice. Allele frequencies were estimated from the intensity scores obtained from all 19 pool assessments by the use of BeadStudio software (version 3.1.00; Illumina Inc). This method was recently proved to be valid in several studies, including case-control studies in late-onset Alzheimer disease[27] and schizophrenia,[28] and a comparison with individual genotyping revealed excellent concordance for the HumanHap300 array.[29] A proximity analysis of these data revealed perfect clustering of the estimated allele frequencies of the 19 pools, which resulted in separate clusters for the 6 phenotypes (early partial response/nonresponse, response/nonresponse after 5 weeks, and remission/nonremission after 5 weeks). The average MAF correlation across pools was 0.98, and the correlation with the individual allele frequencies from the CEU (Utah residents with ancestry from northern and western Europe) sample of the International HapMap Project (http://www.hapmap.org) was 0.93, which matched the result of another European GWAS that used HumanHap arrays with pooled DNA (r=0.94).[28]

We selected 338 SNPs for replication in the STAR*D sample. The selection criteria were the "best" 300 SNPs from the HumanHap300 chip (which corresponded to one-tenth of a percent) that showed concordant associations with treatment outcome in both genomewide samples with the lowest combined P values (geometric mean of the respective P values) plus 38 SNPs from the Sentrix Human-1 chip associated with treatment outcome in the MARS sample, which achieved a $P=1 \times 10^{-4}$. Of these SNPs, 328 could be successfully genotyped using a custom assay (GoldenGate; Illumina Inc), with a call rate greater than 98%. A total of 4.9% of these SNPs showed a nominally significant deviation from HWE at a level of significance of 5%, which corresponded to the expected number of false-positive findings under the null hypothesis of no HWE deviation (P=.96). The average MAF was 27% (range, 7.0%-49.9%), with more than 80% of the SNPs showing an MAF larger than 15%.

POWER CALCULATION

Power calculation was conducted using the CaTS Power Calculator for Genome-Wide Association Studies.[30] Applying a 2-stage design with genomewide scans as the first stage and a replication of 328 genotypes as the second stage, we calculated a power of 80% to detect genetic effects (allelic model) with a relative risk of 1.60 for SNPs with an MAF of at least 15% and under the assumption of a 33% favorable treatment outcome.

STATISTICAL ANALYSES

Pharmacogenetic analyses were conducted by the use of χ^2 statistics. Treatment outcome was evaluated binary as partial response after 2 weeks and response and remission after 5 weeks. Genotypic (MARS sample) and allelic (MARS, German replication, and STAR*D samples) models were calculated. To reduce false-positive results, we corrected for multiple comparisons by the use of a resampling method with 10 000 permutations in accordance with the approach of Westfall and Young,[31] which considers the dependence structure of the genotypes to control for an irregular increase in the β error.

In addition, a multilocus survival analysis was performed in the MARS and STAR*D samples. For this analysis, "response"

©2009 American Medical Association. All rights reserved.
Downloaded From: http://jamanetwork.com/ on 05/19/2013

alleles were determined in accordance with the results of the MARS project for each of the 328 SNPs considered for replication in the STAR*D sample. For 18 SNPs, response alleles could not be unambiguously identified; these SNPs were omitted from the multilocus analysis. We calculated a second score after weighting the number of alleles with the respective odds ratio (OR) from the MARS sample. Cox regression modeling was applied by the use of a proportional hazard function for occurrence and time until remission during the first 8 weeks of treatment. Missing HAM-D and QIDS-C scores were estimated using nonlinear regression to benefit from a complete data set, and HAM-D values from the MARS sample were translated into equivalent QIDS-C scores. With the assumption that a certain threshold of risk alleles may be required to predict an unfavorable outcome, we defined a threshold model of multiple genetic effects. Patients were categorized as high or low response allele carriers according to their additive and weighted response allele scores, respectively. In addition, clinical predictors of treatment outcome, including age at onset; diagnosis of recurrent depression, chronic depression, or a comorbid anxiety disorder (general anxiety disorder, panic disorder, or social phobia); and age and sex, were considered in the Cox regression model. According to previous results of the MARS and STAR*D studies, we assumed beneficial effects on treatment outcome for female sex,[20] young age,[32] late age at onset,[32,33] absence of recurrent[34] and chronic[31,35] depression episodes, and comorbid anxiety disorders.[1,19,20] We further assumed favorable effects for a high number of response alleles. One-sided *P* values according to the prediction hypotheses are reported.

PATHWAY ANALYSIS

A pathway analysis of genes corresponding to the SNPs selected for replication in the STAR*D sample was performed by the use of the Genomatix BiblioSphere PathwayEdition version 7.16 (Genomatix Software Inc, Ann Arbor, Michigan; http://www.genomatix.de/products/BiblioSphere/). BiblioSphere PathwayEdition is a heuristic method used to summarize available evidence about gene relationships by the systematic extraction and analyzation of the following scientific databases: National Center for Biotechnology Information (NCBI) PubMed, NCBI Entrez Gene, and Genomatix MatBase, a comprehensive transcription factor database. Genes were categorized as related if they were co-cited in the same sentence of an abstract with a functional descriptor in between (evidence level B3). Gene clusters were identified in accordance with the number of co-citations of each pair of genes.

RESULTS

GENOMEWIDE ASSOCIATION ANALYSIS

In accordance with previous pharmacogenetic studies,[11,12] we evaluated early partial response (HAM-D score reduction ≥25%) after 2 weeks and response (HAM-D score reduction ≥50%) and remission (HAM-D score <10) after 5 weeks as antidepressant outcome phenotypes. The genomewide results for the MARS sample are presented in **Figure 1**, which shows the effect of the outcome phenotype with the highest genotypic or allelic association. The largest pharmacogenetic association was found for rs6989467 (early partial response, genotypic model, $P=7.6\times10^{-7}$) (Figure 1), which is located in the 5′ flanking region of the *CDH17* gene on 8q22; several other associations with a nominal $P<1\times10^{-5}$ were found. Using the multivariate Fisher product method (geometric mean of the *P* values) across the three outcome phenotypes, the strongest effect was observed for rs1502174 (dominant-recessive model, $P=8.5\times10^{-5}$), located in the 3′ flanking region of the *EPHB1* gene on 3q22. However, no effect withstood correction for multiple testing.

Before testing replication in the STAR*D sample, we performed another GWAS by the use of pooled DNA from an independent German sample of inpatients with depression. This analysis aimed to identify genotypes concordantly associated with treatment outcome in both samples to reduce the likelihood of false-positive results. The effects observed in the pooled sample were within a somewhat smaller range of *P* values, with the highest association found for rs1912674 (early partial response, $P=8.9\times10^{-7}$), located in the region between the *AK090788* and *PDE10A* genes on 6q21. No effect remained significant after correction for multiple testing.

STAR*D REPLICATION SAMPLE

For the replication analysis in the STAR*D sample, we selected 300 SNPs from the Sentrix HumanHap300 chip that showed concordant allelic associations with treatment outcome in both German samples with the lowest combined *P* values. In addition, 38 SNPs from the Sentrix Human-1 chip with the lowest *P* values in the MARS sample were selected. Of these 338 SNPs, 328 were successfully genotyped by the use of a GoldenGate custom assay (eTable; http://www.archgenpsychiatry.com). The genotypes of these SNPs did not differ between patients (MARS sample) and controls matched for age, sex, and ethnicity after correction for multiple testing ($P_{corrected}>.5$). When evaluating associations with treatment outcome in the STAR*D sample (partial response after 2 weeks, response/remission after 5 weeks, and remission at the end of the first treatment period), 46 SNPs were associated at the nominal level of significance ($P_{nominal}<.05$), which showed allelic effects in the same direction as in the MARS and the German replication samples (eTable [bold entries]). These effects, however, did not withstand correction for multiple testing ($P_{corrected}>.1$).

MULTILOCUS ANALYSIS

Next, we investigated whether the prediction of treatment outcome could be improved if multiple allelic effects were considered simultaneously combined with clinical variables. For this purpose, we generated a multilocus genetic variable that described the individual number of alleles associated with beneficial treatment outcome in the MARS sample, with the assumption of an additive effect of the 328 selected SNPs. For 18 SNPs, response alleles could not be unambiguously identified because only the heterogeneous genotype (the presence of both alleles) was associated with favorable treatment outcome. These SNPs were omitted from the multilocus analysis.

We used a survival analytical approach that evaluated the occurrence of remission during the first 8 weeks of treatment, which is the minimal period recommended for clinical studies with remission as the primary outcome.[36] Age, sex, age at onset, recurrence of episodes, chronic episode (≥2 years), comorbid anxiety disorder, and the response

©2009 American Medical Association. All rights reserved.
Downloaded From: http://jamanetwork.com/ on 05/19/2013

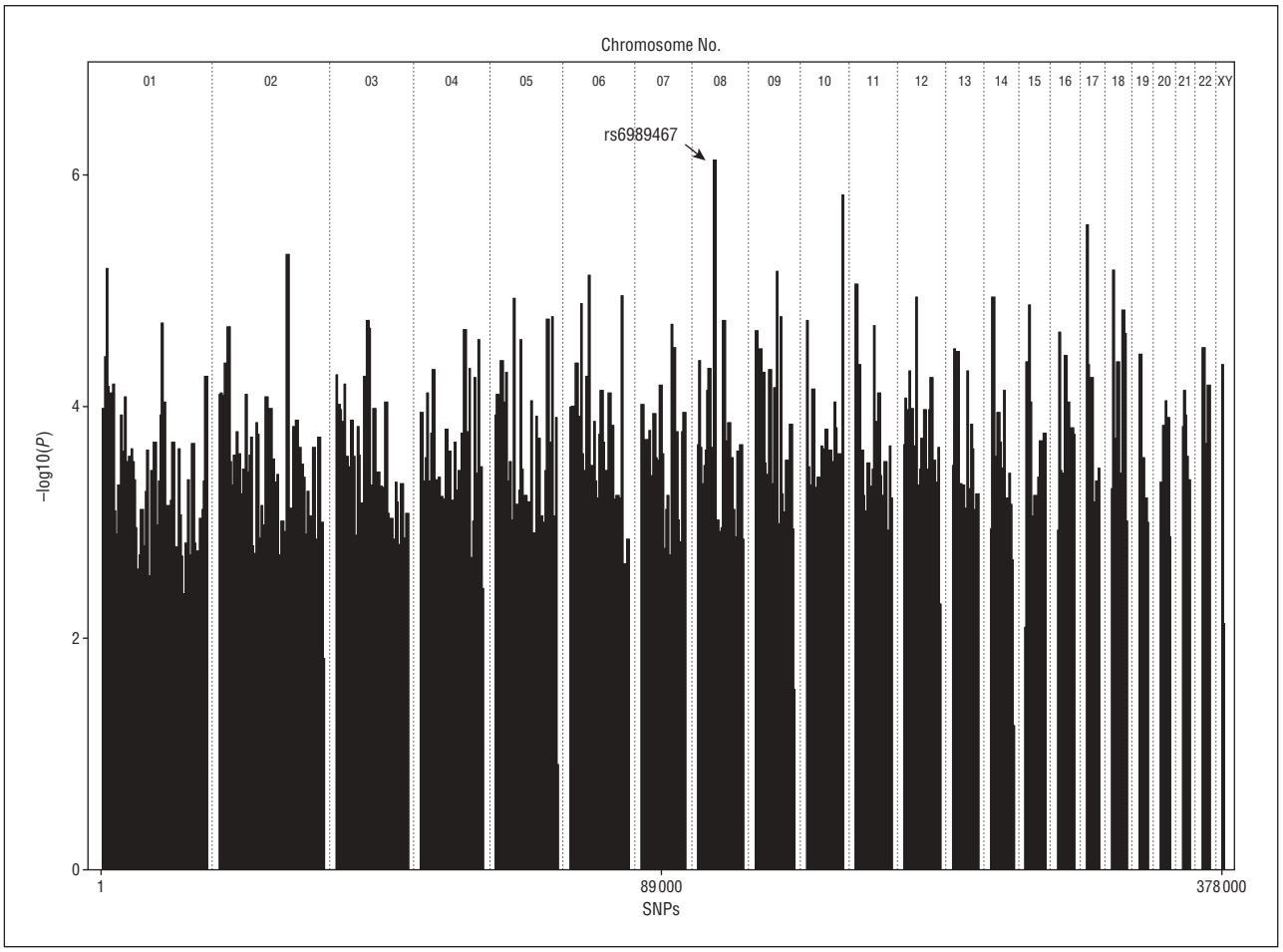

Figure 1. Genomewide pharmacogenetic analysis of early partial response, treatment response, and remission in the Munich Antidepressant Response Signature (MARS) sample. The effect of the outcome phenotype with the highest genotypic or allelic association is presented. The highest genomewide effect was found for rs6989467, located in the 5′ flanking region of the *CDH17* gene on 8q22 (displayed as the negative decadic logarithm of the *P* value). SNP indicates single-nucleotide polymorphism.

allele score were included to predict remission during the first 8 weeks of treatment (**Table 2**).

Consistent for both samples, MARS and STAR*D, and for the combined analysis, the survival analysis demonstrated a negative effect of comorbid anxiety disorder and a positive effect of the number of response alleles, which was significant for the MARS sample ($P=2\times10^{-19}$) and the combined analysis ($P=1\times10^{-16}$) but only approached significance in the STAR*D sample ($P=.08$). We additionally calculated a score after weighting the number of response alleles with the respective OR from the MARS sample. Using this score, we replicated the findings, with the weighted number of response alleles now reaching significance also in the STAR*D sample (OR, 1.01; lower 95% confidence interval, 1.001; $P=.04$).

In accordance with a threshold model of multiple genetic effects, we additionally categorized patients as high or low response allele carriers according to their response allele score. The response allele score ranged from 253 to 361 in the MARS sample. Only one-third of the MARS patients achieved remission during the first 8 weeks. Considering this asymmetry, the cutoff value for defining the response allele carrier status was set accordingly at 320.5, which resulted in 33.3% of patients in the MARS sample being categorized as high response allele carriers, which reflected the base rate of remission. The same threshold was applied for the STAR*D sample. The results of the survival analysis, including the same set of clinical predictors, are given in **Table 3**.

Consistent effects across all samples were again observed for comorbid anxiety disorder (negative effect; approaching significance in the MARS sample, $P=.055$) and for the binary score of high vs low response alleles (positive effect; MARS: $P=1\times10^{-14}$; STAR*D: $P=.04$; combined analysis: $P=7\times10^{-12}$). In addition, a consistent effect was found for young age, which reached significance only in the combined sample. These findings also could be replicated for the analysis with the binary score derived from the weighted number of response alleles.

We additionally defined a binary response allele score based on the reduced set of 46 SNPs that showed nominal significance in the STAR*D sample. The OR for this response allele score was 2.31 ($P=5\times10^{-8}$) in the MARS sample and 1.90 ($P=5\times10^{-9}$) in the STAR*D sample. Comorbid anxiety disorder again displayed a negative effect (MARS: OR, 0.47; $P=.01$; STAR*D: OR, 0.70; $P=.01$). **Figure 2** shows that the best outcome was observed in patients with a high number of response alleles without comorbid anxiety disorder, whereas the worst progno-

©2009 American Medical Association. All rights reserved.
Downloaded From: http://jamanetwork.com/ on 05/19/2013

Table 2. Cox Regression Results That Predict Remission (QIDS-C Score ≤6) During the First 8 Weeks of Antidepressant Drug Treatment, Including Clinical Characteristics and the "Response" Allele Score as Predictors[a]

	Model		Sex[b]		Age		Age at Onset		Recurrent Episode[c]		Chronic Episode[c]		Comorbid Anxiety[c]		Response Allele Score	
	χ^2_7	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
MARS	99.09	2×10^{-19}	0.88 (1.14)	NS	0.99 (1.01)	NS	0.99 (1.01)	NS	1.17 (0.85)	NS	0.79 (1.32)	NS	0.50 (0.89)	.02	1.04 (1.03)	2×10^{-19}
STAR*D	14.64	.041	1.22 (1.01)	.045	0.99 (1.01)	NS	0.99 (1.01)	NS	0.96 (1.27)	NS	1.14 (0.91)	NS	0.67 (0.87)	.006	1.01 (1.00)	.08
Combined	83.84	1×10^{-16}	1.06 (0.91)	NS	0.99 (0.99)	.047	1.01 (0.99)	NS	1.04 (0.84)	NS	1.04 (0.85)	NS	0.61 (0.78)	<.001	1.02 (1.02)	1×10^{-16}

Abbreviations: CI, confidence interval; MARS, Munich Antidepressant Response Signature; NS, not significant; OR, odds ratio; QIDS-C, clinician rating version of the Quick Inventory of Depressive Symptomatology; STAR*D, Sequenced Treatment Alternatives to Relieve Depression.

[a] One-sided 95% CIs of the OR and 1-sided *P* values in favor of the prediction hypothesis are reported.

[b] Sex is coded as "1" for male and as "2" for female patients.

[c] Recurrent (≥1 previous depressive episode) or chronic (duration of current episode ≥2 years) depression and comorbid anxiety (general anxiety disorder, panic disorder, or social phobia) are coded as "1" for true and as "0" for false. One-sided *P* values in favor of the prediction hypothesis are reported.

Table 3. Cox Regression Results That Predict Remission (QIDS-C Score ≤6) During the First 8 Weeks of Antidepressant Drug Treatment, Including Clinical Characteristics and the Binary Score of High vs Low Number of "Response" Alleles as Predictors[a]

	Model		Sex[b]		Age		Age at Onset		Recurrent Episode[c]		Chronic Episode[c]		Comorbid Anxiety[c]		High/Low Response Alleles[d]	
	χ^2_7	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
MARS	77.24	1×10^{-14}	0.90 (1.16)	NS	0.99 (1.01)	.05	1.01 (0.99)	NS	1.22 (0.89)	NS	0.82 (1.36)	NS	0.58 (1.02)	.055	3.52 (2.69)	1×10^{-14}
STAR*D	15.97	.025	1.21 (1.00)	.052	0.99 (1.00)	.09	0.99 (1.01)	NS	0.95 (1.27)	NS	1.13 (0.90)	NS	0.68 (0.88)	.006	1.28 (1.02)	.04
Combined	63.16	7×10^{-12}	1.06 (0.91)	NS	0.99 (0.99)	.02	1.01 (0.99)	NS	1.04 (0.84)	NS	1.03 (0.85)	NS	0.64 (0.81)	7×10^{-12}	1.96 (1.67)	7×10^{-12}

Abbreviations: CI, confidence interval; MARS, Munich Antidepressant Response Signature; NS, not significant; OR, odds ratio; QIDS-C, clinician rating version of the Quick Inventory of Depressive Symptomatology; STAR*D, Sequenced Treatment Alternatives to Relieve Depression.

[a] One-sided 95% CIs of the OR and 1-sided *P* values in favor of the prediction hypothesis are reported.

[b] Sex is coded as "1" for male and as "2" for female patients.

[c] Recurrent (≥1 previous depressive episode) or chronic (duration of current episode ≥2 years) depression and comorbid anxiety (general anxiety disorder, panic disorder, or social phobia) are coded as "1" for true and as "0" for false.

[d] The cutoff value for high vs low number of response alleles was set at 66.7% of the quantitative allele score distribution corresponding to the expected number of remitters after 8 weeks (33.3%) according to MARS data.

sis was obtained for patients with a low number of response alleles combined with comorbid anxiety disorder.

PATHWAY ANALYSIS

Because the multilocus analysis suggested that the SNPs selected for replication in the STAR*D sample contribute additively to treatment outcome in both samples, MARS and STAR*D, we included all corresponding genes in a literature-based pathway analysis. The SNPs located in intergenic regions were assigned to the nearest gene, which resulted in 279 unique genes.

Pathway analysis identified 41 genes co-cited in the same sentence with a functional descriptor in-between. These genes could be grouped into 3 clusters that centered on fibronectin 1 (*FN1*, cluster 1), ADAMTS-like 1 (*ADAMTSL1*, cluster 2), and endothelin 1 (*EDN1*, cluster 3) (**Figure 3**).

FN1 from the first cluster encodes a cell surface glycoprotein mainly involved in cell adhesion processes. *FN1* and 5 other genes of this cluster are involved in metabolic pathways. *FN1* is also related to 2 transcription factors, MYBL2 and NR2E1, and with the substrate (*EFNA5*) and receptor (*EPHA5*) genes of ephrin-A5, an important modulator of late-stage nervous system development and differentiation.

ADAMTSL1 in the second cluster encodes a protein characterized by a desintegrin and metalloproteinase with a thrombospondin motif. This cluster also includes potential risk genes for cardiovascular disorders (*CD36, PON2, APOB,* and *PIK3R1*).

EDN1, the center gene of the third cluster, expresses a protein involved in vasoconstriction. Further notable genes are neuregulin 1 (*NRG1*), a glycoprotein that interacts with the NEU/ERBB2 receptor tyrosine kinase, homer homologue 1 (*Drosophila*) (*HOMER1*), a neuronal immediate early gene and modulator of glutamatergic neurotransmission, and the solute carrier family genes *SLC1A2* (glutamate) and *SLC6A11* (γ-aminobutyric acid).

COMMENT

This is the first report of a GWAS of antidepressant drug treatment response performed in patients from the MARS project and in pooled DNA from an independent German replication sample. A set of 328 SNPs highly related to outcome in both samples was genotyped in a third sample from the STAR*D study. Despite the inclusion of more than 1500 patients with depression, 700 of them with genomewide genotyping, we could not identify single SNP signals that reached the criteria for genomewide significance, which suggests that the effects of single SNPs are rather modest.

©2009 American Medical Association. All rights reserved.
Downloaded From: http://jamanetwork.com/ on 05/19/2013

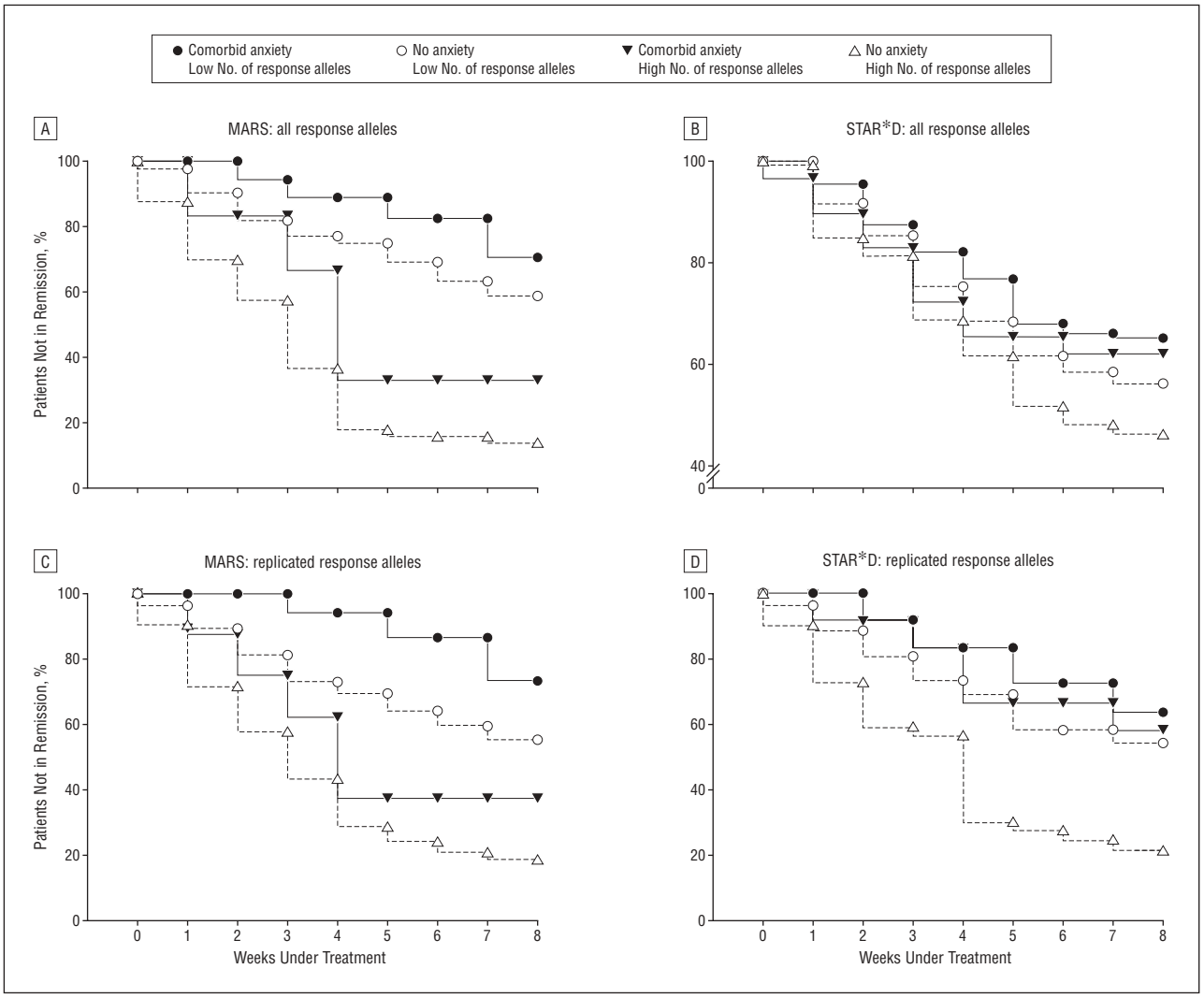

Figure 2. Effects of comorbid anxiety disorder plus high vs low number of response alleles in the Munich Antidepressant Response Signature (MARS) and Sequenced Treatment Alternatives to Relieve Depression (STAR*D) samples. Patients who carry a high number of response alleles (top 33% of the allele score distribution) without comorbid anxiety disorder showed the fastest remission (clinician rating version of the Quick Inventory of Depressive Symptomatology [QIDS-C] score or Hamilton Depression Rating Scale score equivalent of the QIDS-C score of ≤6) in both samples. Survival analysis revealed a large effect, with odds ratios of 3.5 (all 310 single-nucleotide polymorphisms [SNPs]) (A) and 2.3 (46 SNPs with nominal replication) (C) in the MARS sample and of 1.3 (B) and 1.9 (D), respectively, in the STAR*D sample.

Against the backdrop of stringent statistical methods, this analysis provides experimental evidence that antidepressant drug response emerges from a multitude of genetic variants. We constructed a genotype score with the number of favorable response alleles per patient of the set of 310 informative SNPs genotyped in all patients. This multilocus approach revealed a significant contribution of a binary variable that categorized patients as carriers of a high vs low number of response alleles in the prediction of antidepressant drug treatment outcome in both samples (MARS and STAR*D). This finding could be replicated after the weighting of the response allele score for the individual contribution of each allele. In addition, we explored the predictive effect of clinical characteristics when combined with genotype scores. We observed that patients with a comorbid anxiety disorder combined with a low number of response alleles showed the least favorable outcome in the defined observation period. An interaction analysis showed that both effects, comorbid anxiety and the number of response alleles, were independent of each other (data not shown). In fact, this finding is in line with the clinical observation of a tendency toward treatment resistance in the presence of comorbid anxiety disorders.[19]

A literature-based pathway analysis of functional cocitations that includes the genes that correspond to the SNPs of the response allele score revealed a network of 41 genes that could be grouped into 3 interrelated clusters. The first cluster included the transcription factor nuclear receptor subfamily 2, group E, member 1 (*NR2E1*). Variations in this gene have been reported to be associated with susceptibility to bipolar disorder and schizophrenia,[37] and mice that lack this receptor display behavioral abnormalities and impaired neuronal and synaptic plasticity.[38] This cluster also includes the substrate (*EFNA5*) and receptor (*EPHA5*) genes of ephrin-A5, an important modulator of nervous system development and differentiation. This finding is of note because

©2009 American Medical Association. All rights reserved.
Downloaded From: http://jamanetwork.com/ on 05/19/2013

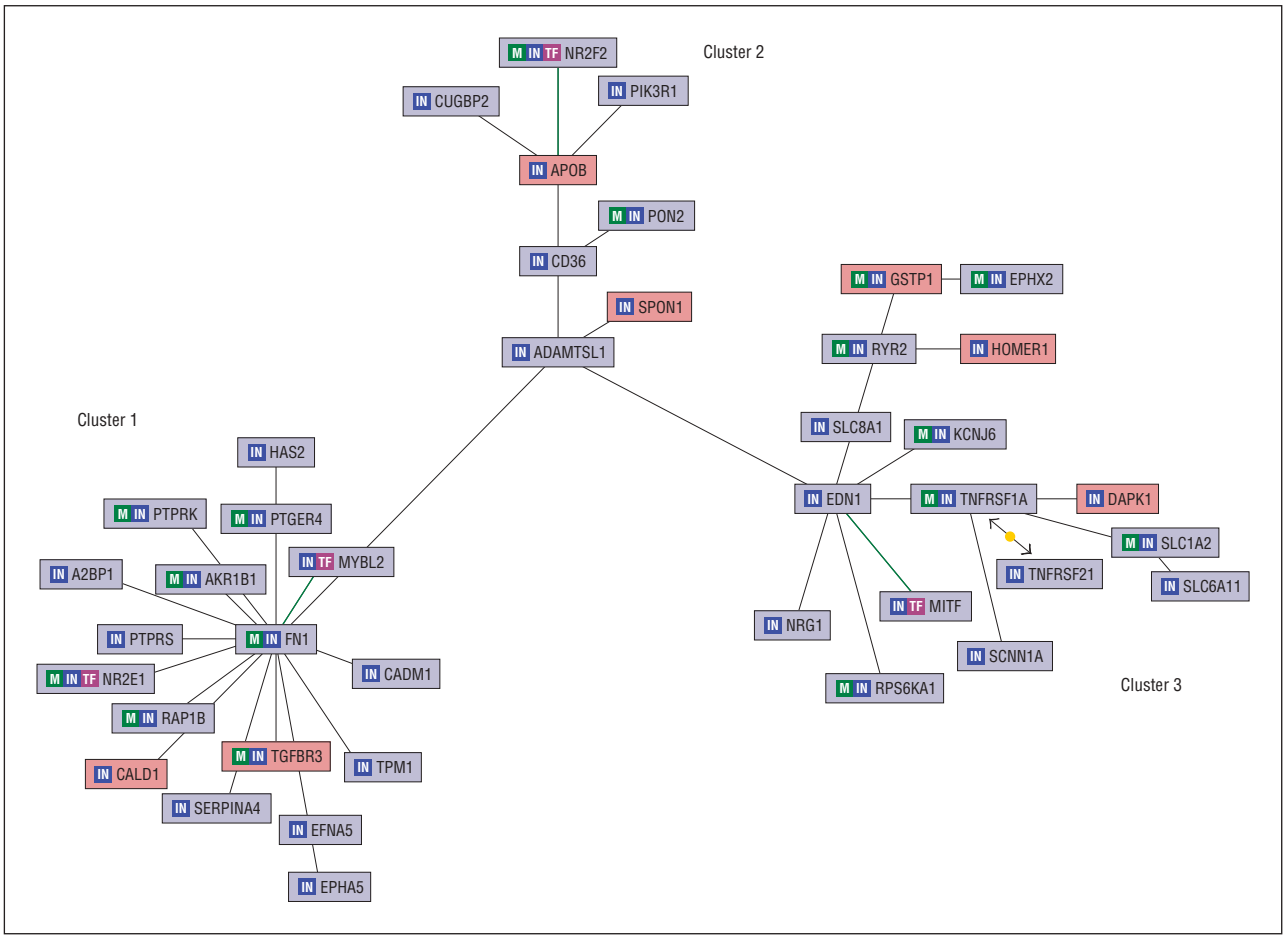

Figure 3. Results of a literature-based pathway analysis that includes all genes that correspond to the single nucleotide polymorphisms (SNPs) of the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) replication sample. Genes were categorized as related when they were co-cited in the same sentence with a functional descriptor in between. We identified 41 genes that cluster around fibronectin 1 (*FN1*) (cluster 1), ADAMTS-like 1 (*ADAMTSL1*) (cluster 2), and endothelin 1 (*EDN1*) (cluster 3). Genes with corresponding SNPs that achieved nominal significant replication in the STAR*D sample are shaded in red; green lines indicate transcription factor (TF) binding site matches in target promoters; the line with the yellow circle indicates annotation by Molecular Connections experts. IN indicates input gene; M, part of a metabolic pathway.

the strongest effect with a combined phenotype of treatment outcome in the MARS sample was observed with an SNP located downstream of *EPHB1*, another receptor from the ephrin family. Studies with mouse mutants demonstrated that the ephrin system regulates the neural plasticity in the hippocampus, a brain area in which adult neurogenesis is stimulated by antidepressant agents.[39]

The second gene cluster identified in the pathway analysis includes genes related to metabolic and cardiovascular disorders that frequently co-occur with depression.[40] Potentially important findings emerged also from the third gene cluster. This cluster includes neuregulin 1 (*NRG1*), for which many genetic studies suggested involvement in the development of schizophrenia[41,42] and bipolar disorder,[43] and presumably also of unipolar depression.[44] Genes of this cluster are related to glutamatergic (homer homologue 1, *HOMER1*; glial high-affinity glutamate transporter, *SLC1A2*) and GABAergic neurotransmission (γ-aminobutyric acid neurotransmitter transporter, *SLC6A11*). Mice that undergo long-term stress treatment[45] or with increased stress susceptibility (M. Schmidt, PhD, Max Planck Institute of Psychiatry, oral communication, August 14, 2008) that model specific features of depressionlike abnormalities displayed altered regulation of *HOMER1* expression in the hippocampal and cortical regions, and rats displayed altered hypothalamic *HOMER1* expression after antidepressant drug treatment.[46] We infer from this pathway analysis that different genetic clusters contribute to treatment outcome in depression, in a manner seemingly related to metabolic pathways and brain development (cluster 1), somatic disability (cluster 2), and receptor signaling and neurotransmission (cluster 3).

Although we included altogether more than 1500 patients, we could not replicate the pharmacogenetic effects of single SNPs. The power analysis suggested sufficient power to detect single effects with a relative genetic risk of 1.6. It seems, however, that the effect size of single SNPs to predict antidepressant drug treatment response is lower than expected. This finding challenges the suitability of GWASs for pharmacogenetic studies in complex diseases. Another limiting factor is the heterogeneity of the investigated phenotype. We tried to address this issue by the inclusion of clinical predictors of treatment outcome, but we have to concede that other factors not considered in this analysis, for example, environmental stress and individual drug history, most likely

©2009 American Medical Association. All rights reserved.
Downloaded From: http://jamanetwork.com/ on 05/19/2013

contributed to the heterogeneity of the phenotype. Nevertheless, we replicated the additive effects of a clinical predictor and a multilocus response allele score. The inclusion of patients in the GWAS samples with bipolar depression may be regarded as a confounder. However, we did not detect differences between patients with unipolar or bipolar depression with respect to the genotype frequencies of the 328 SNPs selected for replication in the STAR*D sample ($P_{corrected} > .48$; data not shown). In addition, the results of multilocus survival analysis suggested that the diagnosis of unipolar vs bipolar depression has no effects on treatment outcome ($P > .33$; data not shown). A further limitation is the heterogeneity of antidepressant drug treatments in the GWAS samples. However, the primary mode of action of all antidepressant agents is related to an enhancement of monoaminergic neurotransmission, and, despite differences in the profile of receptor occupancy, antidepressant drugs show comparable efficacy across drug classes.[47,48] Therefore, we submit that drug-specific genetic effects should be of minor importance for a genomewide pharmacogenetic study.

The present results demonstrate the importance of multiple genetic factors in the prediction of antidepressant drug response, which underscores the multifactorial nature of this trait. In particular, these findings imply a cumulative effect of genetic variations and clinical features. Both types of variables contributed similar effects with respect to prediction of treatment outcome. Further studies are required to confirm the suggested multilocus approach and to investigate the ways that genetic variations and environmental factors converge in a set of genotypes, biomarkers, and clinical features that fosters the decision-making process in the treatment of depression.

Submitted for Publication: November 10, 2008; final revision received January 23, 2009; accepted February 23, 2009.
Author Affiliations: Max Planck Institute of Psychiatry, Munich, Germany (Drs Ising, Lucae, Binder, Bettecken, Uhr, Ripke, Hennings, Horstmann, Kloiber, Menke, Holsboer, and Müller-Myhsok and Mr Kohli); Department of Psychiatry, Ludwig Maximilians University, Munich (Drs Bondy and Rupprecht); Department of Psychiatry, Westfalian Wilhelms University, Muenster, Germany (Drs Domschke, Baune, and Arolt); Department of Psychiatry, James Cook University, Townsville, Australia (Dr Baune); Department of Psychiatry, University of Texas Southwestern Medical Center, Dallas (Dr Rush); and Department of Clinical Sciences, Duke–National University of Singapore (Dr Rush).
Correspondence: Florian Holsboer, MD, PhD, Max Planck Institute of Psychiatry, Kraepelinstrasse 2-10, 80804 Munich, Germany (holsboer@mpipsykl.mpg.de).
Financial Disclosure: None reported.
Funding/Support: The MARS project and genomewide genotyping were supported by the Bavarian Ministry of Commerce and by the Excellence Foundation for the Advancement of the Max Planck Society. Data and sample collection of the STAR*D study were funded by the National Institute of Mental Health, National Institutes of Health, under contract N01MH90003 to the University of Texas Southwestern Medical Center at Dallas (Dr Rush).
Additional Information: The eTable is available at http://www.archgenpsychiatry.com.
Additional Contributions: Michael Czisch, PhD, Tatjana Dose, MD, Peter Lichtner, PhD, Roselind Lieb, PhD, Hildegard Pfister, Benno Pütz, PhD, Philipp Sämann, MD, Daria Salyakina, PhD, Juliane Winkelmann, MD, Thomas C. Baghai, MD, and Cornelius Schüle, MD, provided valuable help in performing the studies; Sabine Damast, Maik Koedel, Susann Sauer, and Alina Tontsch provided technical assistance; the research teams at the BKH Augsburg (Max Schmauß) and the Zentrum für psychische Gesundheit at the Klinikum Ingolstadt (Thomas Pollmächer) contributed cases to the MARS project; the STAR*D research team, especially Maurizio Fava and Madhukar H. Trivedi, acquired clinical data and DNA samples; and Forest Laboratories provided citalopram at no cost for the STAR*D study.

REFERENCES

1. Hennings JM, Owashi T, Binder EB, Horstmann S, Menke A, Kloiber S, Dose T, Wollweber B, Spieler D, Messer T, Lutz R, Künzel H, Bierner T, Pollmächer T, Pfister H, Nickel T, Sonntag A, Uhr M, Ising M, Holsboer F, Lucae S. Clinical characteristics and treatment outcome in a representative sample of depressed inpatients: findings from the Munich Antidepressant Response Signature (MARS) project. *J Psychiatr Res*. 2009;43(3):215-229.
2. Rush AJ, Trivedi MH, Wisniewski SR, Nierenberg AA, Stewart JW, Warden D, Niederehe G, Thase ME, Lavori PW, Lebowitz BD, McGrath PJ, Rosenbaum JF, Sackeim HA, Kupfer DJ, Luther J, Fava M. Acute and longer-term outcomes in depressed outpatients requiring one or several treatment steps: a STAR*D report. *Am J Psychiatry*. 2006;163(11):1905-1917.
3. Roden DM, George AL Jr. The genetic basis of variability in drug responses. *Nat Rev Drug Discov*. 2002;1(1):37-44.
4. Weinshilboum R. Inheritance and drug response. *N Engl J Med*. 2003;348(6):529-537.
5. Angst J. Effect of antidepressants and genetic factors [in German]. *Arzneimittelforschung*. 1964;14:496-500.
6. Franchini L, Serretti A, Gasperini M, Smeraldi E. Familial concordance of fluvoxamine response as a tool for differentiating mood disorder pedigrees. *J Psychiatr Res*. 1998;32(5):255-259.
7. O'Reilly RL, Bogue L, Singh SM. Pharmacogenetic response to antidepressants in a multicase family with affective disorder. *Biol Psychiatry*. 1994;36(7):467-471.
8. Holsboer F. How can we realize the promise of personalized antidepressant medicines? *Nat Rev Neurosci*. 2008;9(8):638-646.
9. Zanardi R, Benedetti F, Di Bella D, Catalano M, Smeraldi E. Efficacy of paroxetine in depression is influenced by a functional polymorphism within the promoter of the serotonin transporter gene. *J Clin Psychopharmacol*. 2000;20(1):105-107.
10. Hu XZ, Rush AJ, Charney D, Wilson AF, Sorant AJ, Papanicolaou GJ, Fava M, Trivedi MH, Wisniewski SR, Laje G, Paddock S, McMahon FJ, Manji H, Lipsky RH. Association between a functional serotonin transporter promoter polymorphism and citalopram treatment in adult outpatients with major depression. *Arch Gen Psychiatry*. 2007;64(7):783-792.
11. Uhr M, Tontsch A, Namendorf C, Ripke S, Lucae S, Ising M, Dose T, Ebinger M, Rosenhagen M, Kohli M, Kloiber S, Salyakina D, Bettecken T, Specht M, Pütz B, Binder EB, Müller-Myhsok B, Holsboer F. Polymorphisms in the drug transporter gene *ABCB1* predict antidepressant treatment response in depression. *Neuron*. 2008;57(2):203-209.
12. Binder EB, Salyakina D, Lichtner P, Wochnik GM, Ising M, Pütz B, Papiol S, Seaman S, Lucae S, Kohli MA, Nickel T, Künzel HE, Fuchs B, Majer M, Pfennig A, Kern N, Brunner J, Modell S, Baghai T, Deiml T, Zill P, Bondy B, Rupprecht R, Messer T, Köhnlein O, Dabitz H, Brückl T, Müller N, Pfister H, Lieb R, Mueller JC, Lõhmussaar E, Strom TM, Bettecken T, Meitinger T, Uhr M, Rein T, Holsboer F, Muller-Myhsok B. Polymorphisms in *FKBP5* are associated with increased recurrence of depressive episodes and rapid response to antidepressant treatment. *Nat Genet*. 2004;36(12):1319-1325.
13. Kirchheiner J, Lorch R, Lebedeva E, Seeringer A, Roots I, Sasse J, Brockmöller

©2009 American Medical Association. All rights reserved.
Downloaded From: http://jamanetwork.com/ on 05/19/2013

J. Genetic variants in *FKBP5* affecting response to antidepressant drug treatment. *Pharmacogenomics*. 2008;9(7):841-846.
14. Lekman M, Laje G, Charney D, Rush AJ, Wilson AF, Sorant AJ, Lipsky R, Wisniewski SR, Manji H, McMahon FJ, Paddock S. The *FKBP5*-gene in depression and treatment response: an association study in the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) Cohort. *Biol Psychiatry*. 2008;63(12):1103-1110.
15. McMahon FJ, Buervenich S, Charney D, Lipsky R, Rush AJ, Wilson AF, Sorant AJ, Papanicolaou GJ, Laje G, Fava M, Trivedi MH, Wisniewski SR, Manji H. Variation in the gene encoding the serotonin 2A receptor is associated with outcome of antidepressant treatment. *Am J Hum Genet*. 2006;78(5):804-814.
16. Paddock S, Laje G, Charney D, Rush AJ, Wilson AF, Sorant AJ, Lipsky R, Wisniewski SR, Manji H, McMahon FJ. Association of *GRIK4* with outcome of antidepressant treatment in the STAR*D cohort. *Am J Psychiatry*. 2007;164(8):1181-1188.
17. Wong ML, Whelan F, Deloukas P, Whittaker P, Delgado M, Cantor RM, McCann SM, Licinio J. Phosphodiesterase genes are associated with susceptibility to major depression and antidepressant treatment response. *Proc Natl Acad Sci U S A*. 2006;103(41):15124-15129.
18. Wong ML, Dong C, Maestre-Mesa J, Licinio J. Polymorphisms in inflammation-related genes are associated with susceptibility to major depression and antidepressant response. *Mol Psychiatry*. 2008;13(8):800-812.
19. Fava M, Rush AJ, Alpert JE, Balasubramani GK, Wisniewski SR, Carmin CN, Biggs MM, Zisook S, Leuchter A, Howland R, Warden D, Trivedi MH. Difference in treatment outcome in outpatients with anxious versus nonanxious depression: a STAR*D report. *Am J Psychiatry*. 2008;165(3):342-351.
20. Trivedi MH, Rush AJ, Wisniewski SR, Nierenberg AA, Warden D, Ritz L, Norquist G, Howland RH, Lebowitz B, McGrath PJ, Shores-Wilson K, Biggs MM, Balasubramani GK, Fava M; STAR*D Study Team. Evaluation of outcomes with citalopram for depression using measurement-based care in STAR*D: implications for clinical practice. *Am J Psychiatry*. 2006;163(1):28-40.
21. Rush AJ, Trivedi MH, Ibrahim HM, Carmody TJ, Arnow B, Klein DN, Markowitz JC, Ninan PT, Kornstein S, Manber R, Thase ME, Kocsis JH, Keller MB. The 16-Item Quick Inventory of Depressive Symptomatology (QIDS), clinician rating (QIDS-C), and self-report (QIDS-SR): a psychometric evaluation in patients with chronic major depression. *Biol Psychiatry*. 2003;54(5):573-583.
22. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders (Fourth Edition, text revision)*. Washington, DC: American Psychiatric Association; 2000.
23. Hamilton M. A rating scale for depression. *J Neurol Neurosurg Psychiatry*. 1960;23:56-62.
24. Rush AJ, Trivedi M, Fava M. Depression, IV: STAR*D treatment trial for depression. *Am J Psychiatry*. 2003;160(2):237.
25. Zimmerman M, Mattia JI. The Psychiatric Diagnostic Screening Questionnaire: development, reliability and validity. *Compr Psychiatry*. 2001;42(3):175-189.
26. Wittchen H-U, Pfister H. *DIA-X Interviews*. Frankfurt, Germany: Swets & Zeitlinger; 1997.
27. Abraham R, Moskvina V, Sims R, Hollingworth P, Morgan A, Georgieva L, Dowzell K, Cichon S, Hillmer AM, O'Donovan MC, Williams J, Owen MJ, Kirov G. A genome-wide association study for late-onset Alzheimer's disease using DNA pooling. *BMC Med Genomics*. 2008;1:44.
28. Kirov G, Zaharieva I, Georgieva L, Moskvina V, Nikolov I, Cichon S, Hillmer A, Toucheva D, Owen MJ, O'Donovan MC. A genome-wide association study in 574 schizophrenia trios using DNA pooling [published online ahead of print March 11, 2008]. *Mol Psychiatry*. doi:10.1038/mp.2008.33.
29. Macgregor S, Zhao ZZ, Henders A, Nicholas MG, Montgomery GW, Visscher PM. Highly cost-efficient genome-wide association studies using DNA pools and dense SNP arrays. *Nucleic Acids Res*. 2008;36(6):e35.
30. Skol AD, Scott LJ, Abecasis GR, Boehnke M. Joint analysis is more efficient than replication-based analysis for two-stage genome-wide association studies. *Nat Genet*. 2006;38(2):209-213.
31. Westfall PH, Young SS. *Resampling-Based Multiple Testing: Examples and Methods for P-Value Adjustment*. New York, NY: John Wiley & Sons; 1993.
32. O'Leary D, Costello F, Gormley N, Webb M. Remission onset and relapse in depression: an 18-month prospective study of course for 100 first admission patients. *J Affect Disord*. 2000;57(1-3):159-171.
33. Moses T, Leuchter AF, Cook I, Abrams M. Does the clinical course of depression determine improvement in symptoms and quality of life? *J Nerv Ment Dis*. 2006;194(4):241-248.
34. Sargeant JK, Bruce ML, Florio LP, Weissman MM. Factors associated with 1-year outcome of major depression in the community. *Arch Gen Psychiatry*. 1990;47(6):519-526.
35. Keller MB, Klerman GL, Lavori PW, Coryell W, Endicott J, Taylor J. Long-term outcome of episodes of major depression: clinical and public health significance. *JAMA*. 1984;252(6):788-792.
36. Rush AJ, Kraemer HC, Sackeim HA, Fava M, Trivedi MH, Frank E, Ninan PT, Thase ME, Gelenberg AJ, Kupfer DJ, Regier DA, Rosenbaum JF, Ray O, Schatzberg AF; ACNP Task Force. Report by the ACNP Task Force on response and remission in major depressive disorder. *Neuropsychopharmacology*. 2006;31(9):1841-1853.
37. Kumar RA, McGhee KA, Leach S, Bonaguro R, Maclean A, Aguirre-Hernandez R, Abrahams BS, Coccaro EF, Hodgins S, Turecki G, Condon A, Muir WJ, Brooks-Wilson AR, Blackwood DH, Simpson EM. Initial association of *NR2E1* with bipolar disorder and identification of candidate mutations in bipolar disorder, schizophrenia, and aggression through resequencing. *Am J Med Genet B Neuropsychiatr Genet*. 2008;147B(6):880-889.
38. Christie BR, Li AM, Redila VA, Booth H, Wong BK, Eadie BD, Ernst C, Simpson EM. Deletion of the nuclear receptor *Nr2e1* impairs synaptic plasticity and dendritic structure in the mouse dentate gyrus. *Neuroscience*. 2006;137(3):1031-1037.
39. Yamaguchi Y, Pasquale EB. Eph receptors in the adult brain. *Curr Opin Neurobiol*. 2004;14(3):288-296.
40. Wells KB, Rogers W, Burnam MA, Camp P. Course of depression in patients with hypertension, myocardial infarction, or insulin-dependent diabetes. *Am J Psychiatry*. 1993;150(4):632-638.
41. Mei L, Xiong WC. Neuregulin 1 in neural development, synaptic plasticity and schizophrenia. *Nat Rev Neurosci*. 2008;9(6):437-452.
42. Stefansson H, Steinthorsdottir V, Thorgeirsson TE, Gulcher JR, Stefansson K. Neuregulin 1 and schizophrenia. *Ann Med*. 2004;36(1):62-71.
43. Georgieva L, Dimitrova A, Ivanov D, Nikolov I, Williams NM, Grozeva D, Zaharieva I, Toncheva D, Owen MJ, Kirov G, O'Donovan MC. Support for neuregulin 1 as a susceptibility gene for bipolar disorder and schizophrenia. *Biol Psychiatry*. 2008;64(5):419-427.
44. Bertram I, Bernstein H-G, Lendeckel U, Bukowska A, Dobrowolny H, Keilhoff G, Kanakis D, Mawrin C, Bielau H, Falkai P, Bogerts B. Immunohistochemical evidence for impaired neuregulin-1 signaling in the prefrontal cortex in schizophrenia and in unipolar depression. *Ann N Y Acad Sci*. 2007;1096:147-156.
45. Orsetti M, Di Brisco F, Canonico PL, Genazzani AA, Ghi P. Gene regulation in the frontal cortex of rats exposed to the chronic mild stress paradigm: an animal model of human depression. *Eur J Neurosci*. 2008;27(8):2156-2164.
46. Conti B, Maier R, Barr AM, Morale MC, Lu X, Sanna PP, Bilbe G, Hoyer D, Bartfai T. Region-specific transcriptional changes following the three antidepressant treatments electro convulsive therapy, sleep deprivation and fluoxetine. *Mol Psychiatry*. 2007;12(2):167-189.
47. Mathew SJ, Manji HK, Charney DS. Novel drugs and therapeutic targets for severe mood disorders. *Neuropsychopharmacology*. 2008;33(9):2080-2092.
48. Wong ML, Licinio J. From monoamines to genomic targets: a paradigm shift for drug discovery in depression. *Nat Rev Drug Discov*. 2004;3(2):136-151.

©2009 American Medical Association. All rights reserved.
Downloaded From: http://jamanetwork.com/ on 05/19/2013