

# Association of Polymorphisms in Genes Regulating the Corticotropin-Releasing Factor System With Antidepressant Treatment Response

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**Context:** The corticotropin-releasing factor (CRF, or corticotropin-releasing hormone) and arginine vasopressin systems have been implicated in the pathophysiology of anxiety and depressive disorders and response to antidepressant treatment.

**Objective:** To study the association of genetic variants in 10 genes that regulate the CRF and arginine vasopressin systems with treatment response to citalopram in the Sequenced Treatment Alternatives to Relieve Depression (STAR\*D) sample (N=1768).

**Design:** Pharmacogenetic association study derived from the STAR\*D study, a multicenter, prospective, open, 12-week effectiveness trial.

**Setting:** Outpatient primary care and psychiatric clinics.

**Patients:** Individuals with nonpsychotic major depressive disorder for whom DNA was available who were subsequently treated with citalopram hydrobromide for 4 to 12 weeks.

**Intervention:** Flexible doses of citalopram.

**Main Outcome Measure:** Association of genetic polymorphisms in genes encoding the CRF system with response and remission to citalopram treatment at exit visit.

**Results:** One single-nucleotide polymorphism (SNP) (rs10473984) within the *CRHBP* locus showed a significant association with both remission ( $P=6.0 \times 10^{-6}$ ; corrected,  $P=.0026$ ) and reduction in depressive symptoms ( $P=7.0 \times 10^{-7}$ ; corrected,  $P=.00031$ ) in response to citalopram. The T allele of this SNP was associated with poorer treatment outcome in 2 of the 3 ethnic subsamples (African American and Hispanic), despite large differences in minor allele frequency. This association was more pronounced in patients with features of anxious depression ( $P=.008$ ). The nonresponse allele was shown to be associated with overall higher plasma corticotropin levels and more pronounced dexamethasone suppression of corticotropin.

**Conclusions:** These data indicate that a genetic variant within the *CRHBP* locus affects response to citalopram in African American and Hispanic patients, suggesting a role for this gene and for the CRF system in antidepressant treatment response.

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**H**YPOTHALAMIC-PITUITARY-adrenocortical (HPA) system hyperactivity has been the most prominent and well-documented neuroendocrine change associated with major depressive disorder (MDD). This HPA hyperactivity is thought to be integral to the pathogenesis, course, and treatment of this disorder.<sup>1,2</sup> Hyperactivity of the corticotropin-releasing factor (CRF; also termed *corticotropin-releasing hormone* [CRH]) and arginine vasopressin (AVP) neuronal systems seem to play a seminal role in the etiology and symptomatology of mood and anxiety disorders.<sup>3-13</sup> In addition, considerable evidence suggests that normalization of the HPA system might be the final step nec-

essary for stable remission of MDD. Successful antidepressant treatment has been shown to attenuate and normalize HPA-axis abnormalities.<sup>14-18</sup> Finally, an open-label study suggested potential clinical efficacy for a CRH receptor type 1 antagonist in depressed patients,<sup>19</sup> but findings have not been consistently replicated.<sup>20</sup>

Given these data, it is likely that genetic polymorphisms that affect the function of genes that regulate this system may modulate an individual's response to antidepressant treatment. Indeed, 3 genes within the HPA-axis system have been reported to be associated with antidepressant response. Licinio et al<sup>21</sup> reported an association of a 3-SNP haplotype within *CRHR1* with response to desipramine or fluoxetine, which was replicated in a Chi-

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nese sample.<sup>22</sup> Interestingly, in both samples, the association of *CRHR1* variants was most pronounced in patients with anxious depression. Binder et al<sup>23</sup> found an association of genetic variants within the gene encoding the glucocorticoid receptor–regulating cochaperone of hsp90, *FKBP5*, with response to antidepressant treatment. This has been replicated in the Sequenced Treatment Alternatives to Relieve Depression (STAR\*D) sample and a German sample.<sup>24,25</sup> Finally, van Rossum et al<sup>26</sup> described carriers of a rare functional polymorphism in the glucocorticoid receptor gene (*ER22/23EK*) as showing a faster response to antidepressant treatment.

The aim of this study was to investigate the association of polymorphisms in genes of the CRH and AVP system with remission and response to citalopram hydrobromide treatment in the STAR\*D sample. To this goal, we genotyped tagging single-nucleotide polymorphisms (SNPs) in the genes encoding the ligands (*UCN*, *UCN2*, *UCN3*, and *CRH*), receptors (*CRHR1* and *CRHR2*), and binding protein (*CRHBP* [OMIM \*122559]) of the CRH system as well as the ligand (*AVP*) and the 2 behaviorally relevant receptors (*AVPR1B* and *AVPR1A*) of the AVP system.

## METHODS

### SAMPLE

The rationale, methods, and design of the STAR\*D study have been detailed elsewhere.<sup>27</sup> In brief, investigators at 14 regional centers across the United States implemented a standard study protocol at 41 clinical sites.

Subjects provided separate written informed consent for study participation and for the collection of blood samples for genetic studies. Outpatients aged 18 to 75 years with a baseline Hamilton Depression Rating Scale score<sup>28</sup> of 14 or higher who met DSM-IV<sup>29</sup> criteria for nonpsychotic MDD were eligible as long as the treating clinician deemed antidepressant medication use to be appropriate. Exclusion criteria are summarized in the supplementary “Methods” section (available at <http://www.archgenpsychiatry.com>). The 16-item Quick Inventory of Depressive Symptomatology–Clinician-Rated (QIDS-C)<sup>30</sup> score was obtained at baseline and at each treatment visit to measure symptom severity. This study only investigates treatment outcomes from the first treatment step (level 1) in STAR\*D, which required an adequate dose of citalopram.<sup>31</sup> Citalopram was given for up to 12 weeks, and response was assessed weekly. No concomitant medications were allowed, except for benzodiazepines and sedatives/hypnotics, if needed. Patients could move to the next treatment level if they were nonresponsive at any visit. This point is referred to as the exit visit,<sup>31</sup> with patients either substantially improved or moved to a different treatment within level 2.

### DNA

DNA samples were collected from 1953 participants of the 4041 patients enrolled in STAR\*D. For details on DNA extraction and quality control as well as a detailed comparison of the demographic characteristics of the 1953 patients who consented to providing a blood sample for DNA vs the other 2088 patients in the whole STAR\*D sample, see the study by McMahon et al.<sup>32</sup> The selection criteria of patients with DNA for this analysis are described below.

## PHENOTYPE DEFINITION

For phenotype definitions, we used the criteria for remission at the last visit of level 1 (exit visit), as proposed by McMahon et al. We only included patients who remained in level 1 for at least 4 weeks so that at the exit visit time, patients had at least 4 weeks of treatment and had remained an average of 11.4 weeks (SD, 3.0 weeks) in the study.

Remitters were those who achieved a QIDS-C score of 5 or less at the last treatment visit; nonremitters had a QIDS-C score of 10 or more at the last visit. Those with a final QIDS-C score in the borderline range of 6 to 9 were excluded from analysis. Thus, 38.2% of patients were nonremitters, 43.0% were remitters, and 18.8% were in the borderline range.

Responders achieved at least a 50% reduction in baseline QIDS-C score at the last treatment visit. Nonresponders did not achieve a 50% reduction in baseline QIDS-C score at the last treatment visit. According to this definition, 42.7% were nonresponders and 57.3% were responders.

As a secondary test, change in QIDS-C score from baseline to last visit was tested as a quantitative measure of change in depressive symptoms. Only 1 patient did not have both measures and was excluded from this analysis.

To test for possible interaction with anxious depression status, we used the definition described in the studies by Fava et al,<sup>33,34</sup> with a baseline Hamilton Rating Scale for Depression (17-item scale) anxiety/somatization factor score of 7 or more considered to indicate anxious depression.

### DRUG TOLERABILITY

Similar to McMahon et al,<sup>32</sup> we also controlled for drug tolerability to avoid misclassification of intolerant patients as nonresponders. Subjects were scored as tolerant, probably tolerant, intolerant, or probably intolerant on the basis of an algorithm that considered study exit data and the Global Rating of Side Effect Burden.<sup>35</sup> (More detail is available in the supplementary “Methods” section.)

### CANDIDATE GENES AND SNP SELECTION

Ten genes involved in the regulation of the AVP or CRH system were chosen. **Table 1** lists the genes by chromosomal position and the number of selected SNPs. One hundred seventeen tagging SNPs were selected, as presented in the online Appendix.

### SNP GENOTYPING

Using the online tool provided by Applied Biosystems (ABLaunch-SNPlex), 3 multiplexed SNP panels were designed for the SNPlex platform (Applied Biosystems) from 113 SNPs. Five SNPs failed the assay design algorithm (SNP panels could not be designed for 2 SNPs in *AVP*, 2 in *UCN2*, and 1 in *CRHR1*). A total of 108 SNPs were thus genotyped on the SNPlex platform using a standard protocol at the genetic core facility at University of California–Los Angeles. Four more SNPs, all in *CRHR1*, were genotyped using predesigned TaqMan assays (Applied Biosystems) on an ABI7900 HT Real Time PCR Instrument (Applied Biosystems). Overall, 76 (68%) SNPs reached a call rate greater than 90% in this initial experiment. Nearly 16% (15.7%) had a call rate less than 50%, and the average call rate of all the SNPs was 82%. A set of 30 DNA samples of the 1863 genotyped on SNPlex failed more than 80% of all SNPlex assays, indicating a potential problem with DNA quantity or solubility in this assay. The corrected average call rate of the SNPs reaching at least 90% is thus 94.8%. All 4 TaqMan assays were genotyped in 1897 individuals with a call

**Table 1. Selected Candidate Genes and Number of Selected Tagging SNPs**

Gene	Gene Product Name	RefSeq Identification	Position on Chromosome	No. of Selected SNPs	No. of Exons	Gene Size, Bases
<i>AVP</i>	Arginine vasopressin	NM_000490	20p13	8	3	2168
<i>AVPR1A</i>	Arginine vasopressin receptor 1A	NM_000706	12q14-q15	6	2	6375
<i>AVPR1B</i>	Arginine vasopressin receptor 1B	NM_000707	1q32	14	2	7688
<i>CRH</i>	Corticotropin-releasing hormone	NM_000756	8q13	17	2	2080
<i>CRHBP</i>	Corticotropin-releasing hormone binding protein	NM_001882	5q11.2-13.3	13	7	16 619
<i>CRHR1</i>	Corticotropin-releasing hormone receptor 1	NM_004382	17q12-q22	19	13	51 525
<i>CRHR2</i>	Corticotropin-releasing hormone receptor 2	NM_001883	7p15.1	21	12	29 278
<i>UCN</i>	Urocortin	NM_003353	2p23-p21	5	2	866
<i>UCN2</i>	Urocortin 2/stresscopin-related peptide	NM_033199	3p21.3	4	2	2049
<i>UCN3</i>	Urocortin 3/stresscopin	NM_053049	10p15.1	5	2	9194

Abbreviations: RefSeq, National Center for Biotechnology Information Reference Sequence; SNP, single-nucleotide polymorphism.

rate ranging from 99.05% to 99.84%, with 100% concordance of 352 duplicate genotypes. Hardy-Weinberg equilibrium was examined separately for the 3 ethnic groups represented in STAR\*D (European American, African American, and Hispanic). Three SNPs showed significant deviations from Hardy-Weinberg equilibrium in 2 or more populations and were thus also excluded from the analysis. Table 1 lists the SNP names, gene names, positions on the chromosome, minor allele frequency (MAF), and *P* value for Hardy-Weinberg equilibrium for the 76 SNPs with a call rate greater than 90% that were included in the analysis. The genetic coverage of the SNP selection passing quality-control criteria (call rate  $\geq$  90% and in Hardy-Weinberg equilibrium,  $n=73$ ) is described in the online Appendix.

To control for the possibility of false-positive associations due to genotyping error, we regenotyped the 5 SNPs in *CRHBP*, with the strongest association using TaqMan-based assays. For these, we had 603 duplicate assessments for a total of 14 044 genotypes with 0 discordances. Call rates were over 99% for all 5 SNPs. For the 5 regenotyped SNPs, the discordance rate was 0.028 between SNPlex and TaqMan genotypes.

### DEXAMETHASONE SUPPRESSION

The individuals were recruited within a larger study to investigate the roles of genetic and environmental factors in predicting the development of posttraumatic stress disorder.<sup>36,37</sup> Research participants were approached while in the waiting rooms of primary care or obstetrical-gynecological clinics of Grady Memorial Hospital in Atlanta, Georgia. Informed consent was obtained for all subjects, and all procedures in this study were approved by the institutional review boards of Emory University School of Medicine and Grady Memorial Hospital. In a subsample of 164 individuals of mostly African American descent (91.6% African American), a low-dose dexamethasone suppression test with a 0.5-mg dose was performed as described in the supplementary "Methods" section. Seven of the 164 probands had no detectable dexamethasone levels and were excluded from the analysis. Significance of the interaction effect of risk allele carrier status for rs10473984 (TT and TG vs GG) and depressive symptoms (cutoff of Beck Depression Inventory score  $\geq$  16, with 31.7% classified as currently depressed according to this cutoff) was determined using repeated-measures analysis of variance to examine the main effect of rs10473984 and depressive symptoms status (between-subject factor) and their interaction term on the change in serum cortisol and corticotropin from day 1 to day 2 (within-subject factor). All analyses were covaried for by age, sex, Childhood Trauma Questionnaire total score, and Posttraumatic Stress Disorder Symptom Scale total score.

### STATISTICAL ANALYSIS

We restricted our analysis to patients with at least moderate symptom severity as defined by a baseline QIDS-C score of 10 or more (30 patients had less severe baseline QIDS-C scores) and who remained in level 1 for at least 4 weeks (154 were at level 1 <4 weeks). Of the 1768 patients fulfilling these criteria, DNA was not available for 49 owing to quality-control issues, and another 47 samples were not genotyped in the SNPplex assay owing to 384-well plate layout, and thus cost reasons. This brought the number of patients entering the analysis with SNPplex genotypes to 1672 and for the TaqMan SNPs, to 1719. The initial analysis strategy was to test for allelic and additive genetic (genotypes coded as 0, 1, and 2) associations of the included 73 SNPs with remission and response at exit visit and change in QIDS-C score from baseline to exit visit. The initial analysis was performed in the combined samples, not stratified by race. Analyses were run using logistic or linear regression, adjusted for age and sex. We established the significance of main genetic effects for each SNP using permutation-based procedures that randomly assigned the outcomes (remitter-responder status and change in QIDS-C score) to subjects (sampled without replacement), while holding each subject's genotype fixed. This permutation method is robust against non-normal distribution of the outcome variable and small cell sizes. For each analysis, the empirical *P* value was based on at least 10 000 permutations. We conducted these analyses using appropriate components of the SAS software system (version 9.1; SAS Institute, Cary, North Carolina) as described in previous studies.<sup>36,37</sup>

Secondary analyses then stratified the sample by racial category (European American, African American, and Hispanic) and used estimated proportion of African biogeographical ancestry as an additional covariate.

To assess interaction effects of SNPs with anxious depression on treatment response, we used a repeated-measures general linear model with QIDS score at the baseline and exit visits as the within-subject factor and presence of anxious depression, SNP genotype, and their interaction term as between-subject measures. Age, sex, and estimated proportion of African biogeographical ancestry were used as covariates. These analyses were carried out using SPSS, version 15.0.

For our main SNP analysis, we used Bonferroni correction for multiple testing, adjusting for the number of tested SNPs ( $n=73$ ) and phenotypes (remission, response, and change in QIDS score) as well as genetic model (additive and allelic). The adjusted  $\alpha$  level was thus  $0.05/(73 \times 3 \times 2) = .000114$ . In this article, we present the empirical *P* values as well as the adjusted Bonferroni-corrected *P* values for all analyses in the total sample.

**Table 2. All SNPs Associated With Remission and Response With  $P < .05$  in the Additive Genetic Model From the SNPplex Screening Genotyping**

SNP	Gene	P Value		Allelic OR (95% CI)
		Additive	Allelic	
<b>Remission</b>				
rs10473984	<i>CRHBP</i>	.00044	.00062	1.61 (1.22-2.12)
rs10055255	<i>CRHBP</i>	.0050	.0023	1.26 (1.08-1.41)
rs12942300	<i>CRHR1</i>	.0087	.0015	1.42 (1.14-1.77)
rs10474485	<i>CRHBP</i>	.018	.0062	1.28 (1.07-1.53)
rs2267716	<i>CRHR2</i>	.024	.0098	1.23 (1.05-1.45)
rs6472258	<i>CRH</i>	.029	.045	1.30 (1.01-1.67)
rs7307997	<i>AVPR1A</i>	.047	.038	1.17 (1.01-1.36)
<b>Response</b>				
rs10473984	<i>CRHBP</i>	.0068	.0044	1.42 (1.11-1.81)
rs10474485	<i>CRHBP</i>	.018	.0065	1.25 (1.06-1.46)
rs10055255	<i>CRHBP</i>	.020	.017	1.19 (1.04-1.39)
rs2267716	<i>CRHR2</i>	.024	.013	1.20 (1.04-1.38)
rs12942300	<i>CRHR1</i>	.038	.0086	1.31 (1.07-1.60)
rs255105	<i>CRHR2</i>	.043	.0086	1.20 (1.05-1.38)

Abbreviations: CI, confidence interval; OR, odds ratio; SNP, single-nucleotide polymorphism.

## RACE AND ETHNICITY

Subjects were enrolled into the STAR\*D study without regard to race or ethnicity; 65.5% described themselves as European American, 15.6% as African American, and 14.0% as Hispanic. To control for possible effects of admixture, we used 18 ancestry informative markers to estimate biogeographical ancestry from the 4 continental population groups (Appendix). Using STRUCTURE, version 2.0,<sup>38</sup> and a 4-population model, we calculated the estimated proportion of African ancestry for each individual and used this value as a covariate. Individuals missing genotype data for more than 3 of these markers were excluded from these analyses (8.8%).

## RESULTS

### ASSOCIATION WITH REMISSION AND RESPONSE AT LAST VISIT

Of the 73 tested SNPs, 7 showed nominally significant associations with remission at exit visit (**Table 2**). Of these, 3 SNPs were located in *CRHBP*, including the best SNP, rs10473984. This SNP showed  $P = .00044$  (corrected,  $P > .05$ ) for the additive test and  $P = .00062$  (corrected,  $P > .05$ ) for the allelic test. For the response at the exit visit, 6 SNPs showed nominally significant associations, with the 3 best  $P$  values observed for 3 *CRHBP* SNPs. The best SNP was again rs10473984, with an additive genetic value of  $P = .0067$  (corrected,  $P > .05$ ) and an allelic value of  $P = .0044$  (corrected,  $P > .05$ ). Thus, no SNP withstood correction for multiple testing with this phenotype. Five of the 6 SNPs associated with response were also within the 7 SNPs that showed nominal associations with remission (Table 2).

### CRHBP AND QUANTITATIVE PHENOTYPES

We performed a linear regression with the difference between baseline and exit visit QIDS scores as an outcome

and the 73 SNPs as predictors, adjusting for sex and age. For this phenotype, the 4 most significant  $P$  values were seen for *CRHBP* SNPs. The best association was seen with rs10473984 ( $P = 6.56 \times 10^{-5}$ ; corrected,  $P = .028$ ), followed by rs10055255 ( $P = .0029$ ; corrected,  $P > .05$ ), rs28365143 ( $P = .0056$ ; corrected,  $P > .05$ ), and rs10062367 ( $P = .0145$ ; corrected,  $P > .05$ ). This included the 2 top SNPs for the association with remission (rs10473984 and rs10055255); the 2 other SNPs had not shown significant associations with remitter status.

### DRUG TOLERABILITY

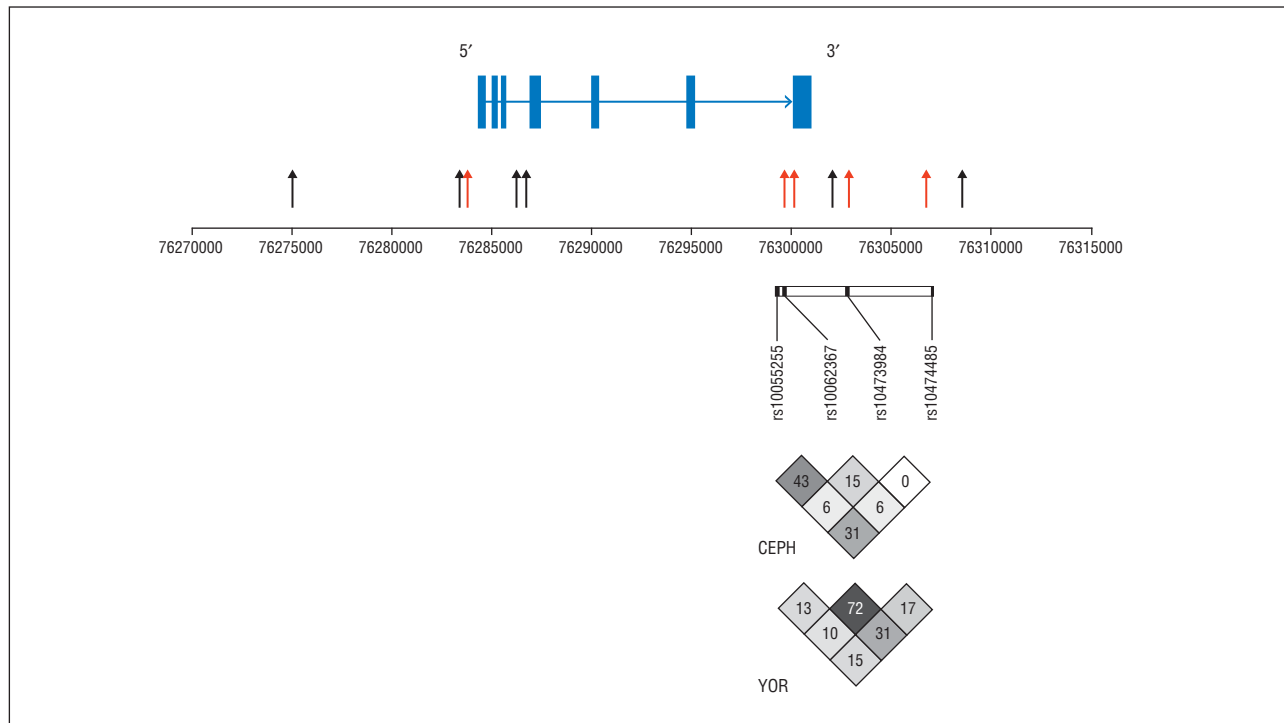
None of the 73 tested SNPs showed a significant association with citalopram tolerability after correcting for multiple testing. For rs10473984, for example, the genotype distribution in tolerant individuals ( $n = 1269$ ) was 85.74% GG, 13.08% GT, and 1.18% TT, and in intolerant individuals ( $n = 151$ ), 82.78% GG, 15.23% GT, and 1.99% TT ( $P = .27$ ).

### POSITION OF ASSOCIATED SNPs ACROSS PHENOTYPES

We observed a clustering of SNPs associated with treatment outcome phenotypes in the *CRHBP* locus, with 4 SNPs at the 3' end of the gene and rs28365143 at the 5' end of the genes. The 4 SNPs at the 3' end of the genes only display moderate linkage disequilibrium (**Figure 1**). Because the only association that withstood correction for multiple testing lies within this locus, we focused the remainder of the experiments on *CRHBP*.

### ANALYSIS USING TaqMan REGENOTYPING DATA FOR THE TOP 5 CRHBP SNPs

To control for possible false-positive associations due to genotyping error, we regenotyped the top 5 SNPs in *CRHBP*



**Figure 1.** Position of single-nucleotide polymorphisms (SNPs) in *CRHBP* that were included in the analysis and linkage disequilibrium structure of 4 SNPs at the 3' end of the gene associated with remission or change in Quick Inventory of Depressive Symptomatology score. The x-axis represents the position on chromosome 5, according to the University of California–Santa Cruz Genome Browser, version hg18. Value for linkage disequilibrium for the 4 associated 3' *CRHBP* SNPs are reported as  $r^2$  values for the Centre d'Etude du Polymorphisme Humain (CEPH) and Yoruban (YOR) populations.

using TaqMan-based assays. Here, the call rates were greater than 99% for all SNPs. Increasing the effective sample size in the analysis also increased the significance of the associations (with rs10473984 now associated at  $P=1.0 \times 10^{-5}$ ; corrected,  $P=.0043$ , additive model; and  $P=6.0 \times 10^{-6}$ ; corrected,  $P=.00043$ , allelic model with remission at exit visit). For response at the exit visit, the respective  $P$  values were now  $P=.0001$ ; corrected,  $P=.043$ ; and  $P=.0006$ ; corrected,  $P>.05$ ; and for the difference in QIDS score from baseline to exit visit,  $P=1.0 \times 10^{-5}$ ; corrected,  $P=.0043$ ; and  $P=7.0 \times 10^{-7}$ ; corrected,  $P=.00031$ . For this SNP, the T allele is associated with poorer response to treatment, with an odds ratio (OR) of 1.88 (95% confidence interval [CI], 1.43–2.46) for remission and 1.29 (95% CI, 1.05–1.59) for response. The associations of the other *CRHBP* SNPs all remained nominally significant, but none passed correction for multiple testing (eTable 2).

#### ANALYSIS USING A SPLIT-SAMPLE DESIGN

We then repeated the analysis for association of these 5 SNPs with remission, response, and change in QIDS-C score in the split-sample design previously described,<sup>32</sup> in which the STAR\*D sample was randomly divided in a discovery ( $n=587$ ) and test ( $n=1132$ ) set. Of the 5 tested *CRHBP* SNPs, only rs10473984 showed associations, with  $P<.01$  in both the discovery and test samples, with  $P=.0091$  and  $P=.00006$  in the additive model for remission at exit visit,  $P=.0003$  and  $P=.0077$  for response, and  $P=.0007$  and  $P=.0002$  for change in QIDS score from admission to exit visit in the discovery and test subsets, respectively. In both subsamples, the T allele was associ-

ated with poorer response (OR, 2.00; 95% CI, 1.22–3.29 in the discovery sample; and OR, 1.30; 95% CI, 1.01–1.68 in the test set for remission at exit visit) (eTable 2).

#### ANALYSIS STRATIFIED BY SELF-IDENTIFIED RACE

All of the 5 *CRHBP*-associated SNPs show significant differences in allele frequencies among the 3 ethnic groups, and poorest response to citalopram treatment overall in African American and Hispanic subjects has been reported.<sup>31</sup> Our results in the combined sample could thus be due to population stratification, with more nonremitters in the 2 minority groups. However, when the analysis was repeated with ethnicity included as a covariate (European American, African American, and Hispanic), the association of rs10473984 with reduction of depressive symptoms remains significant after correction for multiple testing ( $P=2.0 \times 10^{-5}$ ; corrected,  $P=.0087$ ).

eTable 2 shows the  $P$  values for the associations in the 3 subgroups. The associations with rs10473984 that withstood correction for multiple testing and are significant in both sets of the split sample are reported in more detail below (Table 3).

Figure 2 shows the changes in QIDS-C score from baseline to last visit for this SNP in all patients as well as separately for the 3 ethnic groups. None of the associations were significant in European Americans, but in both African American and Hispanic subjects, significant associations (additive model) were observed with remission (African American,  $P=.0076$ , and Hispanic,  $P=.012$ ), response ( $P=.0073$  and  $P=.0097$ , respectively), and

**Table 3. Associations of rs10473984 Regentyped With Remission and Response at Exit Visit**

Outcome	Remission					Response				
	Genotype, No. (%)			P Value	Allelic OR (95% CI)	Genotype, No. (%)			P Value	Allelic OR (95% CI)
	GG	GT	TT			GG	GT	TT		
<b>All Samples</b>										
No remission or response	515 (79.35)	121 (18.64)	13 (2.00)	1.0 × 10 <sup>-5</sup> , <sup>a</sup> 6.0 × 10 <sup>-6b</sup>	1.88 (1.43-2.46)	583 (80.30)	129 (17.77)	14 (1.93)	.0001, <sup>a</sup> 7.0 × 10 <sup>-5b</sup>	1.29 (1.05-1.59)
Remission or response	653 (88.24)	80 (10.81)	7 (0.95)	.0023 <sup>c</sup>		857 (87.27)	115 (11.71)	10 (1.02)	.007 <sup>c</sup>	
<b>European American</b>										
No remission or response	359 (89.97)	40 (10.03)	0	.47, <sup>a</sup> .47 <sup>b</sup>	1.19 (0.77-1.84)	410 (90.51)	43 (9.49)	0	.74, <sup>a</sup> .76 <sup>b</sup>	1.08 (0.72-1.61)
Remission or response	487 (91.89)	41 (7.74)	2 (0.38)	.56 <sup>c</sup>		623 (91.48)	56 (8.22)	2 (0.29)	.96 <sup>c</sup>	
<b>African American</b>										
No remission or response	49 (41.53)	57 (48.31)	12 (10.17)	.0076, <sup>a</sup> .0065 <sup>b</sup>	1.82 (1.17-2.84)	51 (40.80)	61 (48.80)	13 (10.40)	.0073, <sup>a</sup> .0074 <sup>b</sup>	1.69 (1.14-2.49)
Remission or response	54 (60.00)	32 (35.56)	4 (4.44)	.011 <sup>c</sup>		72 (57.60)	46 (36.80)	7 (5.60)	.017 <sup>c</sup>	
<b>Hispanic</b>										
No remission or response	78 (82.11)	17 (17.89)	0	.011, <sup>a</sup> .013 <sup>b</sup>	4.88 (1.40-16.98)	78 (82.11)	17 (17.89)	0	.0097, <sup>a</sup> .66 <sup>b</sup>	4.88 (1.40-16.98)
Remission or response	73 (96.05)	3 (3.95)	0	.0058 <sup>c</sup>		73 (96.05)	3 (3.95)	0	.0014 <sup>c</sup>	

Abbreviations: CI, confidence interval; OR, odds ratio.

<sup>a</sup>For additive model.

<sup>b</sup>For allelic model.

<sup>c</sup>For additive model corrected for African ancestry.

change in QIDS-C score ( $P = .0065$  and  $P = .0002$ , respectively). Of note, the T allele associated with poorer response is rare in European American and Hispanic subjects ( $MAF < 6\%$ ) but relatively common in African Americans (28.1%). While no effect is seen in European Americans (OR, 1.19; 95% CI, 0.77-1.84), there is a poorer response to citalopram treatment in T allele-carrying African American (OR, 1.82; 95% CI, 1.17-2.84) and Hispanic (OR, 4.88; 95% CI, 1.40-16.98) subjects (Figure 2 and Table 3).

#### CONTROLLING FOR ESTIMATED BIOGEOGRAPHICAL AFRICAN ANCESTRY

Because rs10473984 is most common in populations of African descent and the degree of admixture of African ancestry within African American and Hispanic subjects could drive the association, we also controlled for estimated African ancestry using ancestry informative markers. Data describing the performance of the 18 ancestry informative markers are presented in the Appendix.

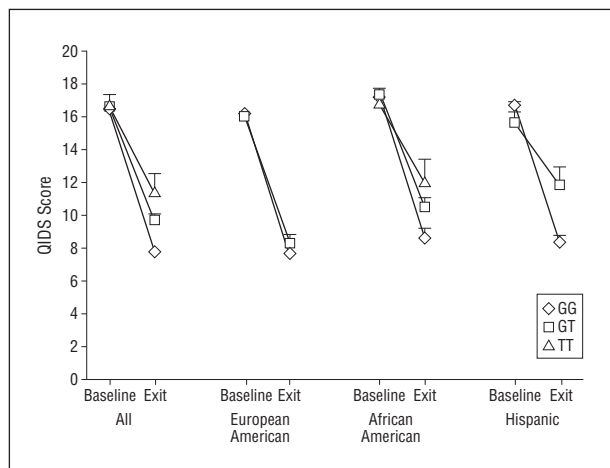
When the association of the *CRHBP* SNPs with treatment outcome was reanalyzed with the estimated proportion of African ancestry as a covariate, in addition to age and sex, associations with remission, response, and change in QIDS-C score remained significant for rs10473984 in the whole sample, as well for the African American and Hispanic subsamples (Table 3). For change in QIDS-C score, the corrected  $P$  value in the additive model was  $P = .0001$  and would thus withstand correction for multiple testing ( $P = .043$ ). See eTable 2 for other SNPs.

#### ANXIOUS DEPRESSION

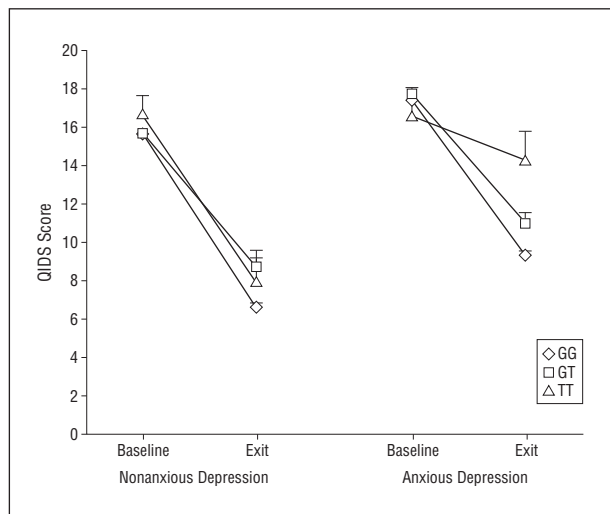
Because of the potential involvement of the CRH system in the pathophysiology of anxious depression, we investigated the interaction of the rs10473984 genotype and anxious depression on treatment response using a repeated-measures analysis on QIDS-C score at baseline and exit visits as the within-subject factor, controlling for age, sex, and estimated African ancestry in the whole sample as well as the combined African American and Hispanic subset. In the analyzed sample, 43% were categorized as having anxious depression. In the whole sample, we observed a significant main effect of rs10473984 genotype and anxious depression status. We also observed an additional significant interaction of change in QIDS-C score from baseline to exit visit with rs10473984 genotype and anxious depression ( $F_{1,421,2} = 4.82$ ,  $P = .008$ ). This interaction term was also significant when restricting the analysis to African American and Hispanic patients combined ( $F_{3,97,2} = 3.67$ ,  $P = .026$ ). In both analyses, the association of the rarer T allele with poorer treatment response was much more pronounced in patients with anxious depression (Figure 3).

#### LOW-DOSE DEXAMETHASONE SUPPRESSION AND rs10473984

In an independent cohort of 157 mostly African American individuals, we tested the effects of rs10473984 T carrier status dependent on current depressive symptoms on low-dose dexamethasone suppression of serum cortisol and corticotropin using a repeated-measures analysis of variance, with day 1 and day 2 cortisol and corti-



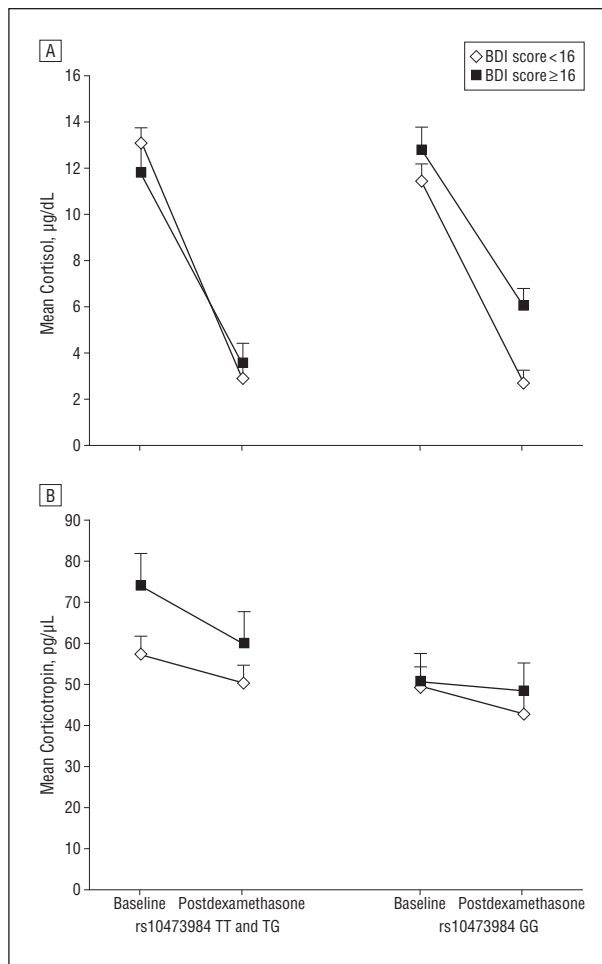
**Figure 2.** Quick Inventory of Depressive Symptomatology (QIDS) scores at baseline and last visits according to rs1043984 in the whole sample and separately for European American, African American, and Hispanic subjects. Allelic  $P$  values were  $P=7.0 \times 10^{-7}$  for the whole sample and  $P=.18$  in European American,  $P=.0064$  in African American, and  $P=.0001$  in Hispanic subjects. Only 2 European Americans carried the TT genotype and were thus excluded from the figure.



**Figure 3.** Quick Inventory of Depressive Symptomatology (QIDS) scores at baseline and last visits according to rs1043984 genotype and anxious depression status. The respective populations in the GG, GT, and TT genotype groups for nonanxious depression are 808, 135, and 11 subjects, and for anxious depression, 617, 104, and 13 subjects.

corticotropin measures as the within-subject factors. For cortisol measures, we observed a significant change in cortisol levels from day 1 to day 2 ( $F_{149,1}=17.3, P<.001$ ), a significant interaction of the change in cortisol and depression symptoms ( $F_{149,1}=4.07, P=.045$ ), a significant main effect of sex ( $F_{149,1}=64.7, P<.001$ ), and a significant interaction of current depressive symptoms and rs10473984 T carrier status ( $F_{149,1}=6.47, P=.012$ ). In fact, depressed carriers of the rs10473984 GG genotype had higher overall cortisol levels compared with the other groups (**Figure 4A**). All other tested main effects and interactions were not significant.

For corticotropin measures, we observed a significant change in corticotropin serum concentration from day 1 to day 2, and significant interaction of the change



**Figure 4.** Mean cortisol (A) and corticotropin (B) serum levels at baseline and following 0.5-mg low-dose dexamethasone suppression according to current depressive symptoms and rs1047984 T allele-carrier status. There were 60 nondepressed rs1047984 T allele carriers, 20 depressed rs1047984 T allele carriers, 48 nondepressed rs1047984 GG carriers, and 29 depressed rs1047984 GG carriers. Error bars represent standard error of the mean; BDI, Beck Depression Inventory.

in corticotropin with sex, age, and total Childhood Trauma Questionnaire score ( $P<.05$  for all). We also observed a significant main effect of rs10473984 T carrier status ( $F_{148,1}=5.36, P=.022$ ), a significant interaction of the change in corticotropin with rs10473984 T carrier status ( $F_{148,1}=4.77, P=.031$ ), and a significant triple interaction of the change in corticotropin with rs10473984, T carrier, and depressive symptom status ( $F_{148,1}=4.27, P=.040$ ). T allele carriers had overall higher corticotropin levels and showed more pronounced suppression following treatment with dexamethasone as a group. The enhanced dexamethasone suppression associated with this group is most pronounced when comparing individuals with a current Beck Depression Inventory total score greater or equal to 16 (**Figure 4B**).

#### COMMENT

In this study, we describe associations of SNPs within genes regulating the CRH system with remission and response to antidepressant treatment in the STAR\*D sample.

The association of 1 SNP located at the 3' end of *CRHBP* (rs10473984), withstanding correction for multiple testing, is significantly associated in the discovery as well as the test set of the STAR\*D sample and remains significant after correction for ethnicity and estimated proportion of African ancestry. This association is restricted to individuals with African American or Hispanic ethnicity, and this result seems to be predominantly carried by individuals with anxious depression. Furthermore, this variant is associated with glucocorticoid-receptor resistance and higher HPA-axis hormone levels. A hyperactive HPA axis at inpatient admission has been reported to correlate with better response to antidepressant therapy in male patients.<sup>39</sup>

Although these results need independent replication, the association of rs10473984 is seen in the discovery as well as the test sets of the STAR\*D sample, using a previously described random split approach.<sup>32</sup> Furthermore, the T allele of this SNP was consistently associated with poorer response to treatment in 2 ethnic subsamples and showed nominally significant associations in the African American and Hispanic subsets of the sample, despite differences in MAF and linkage disequilibrium structure. While not significant in European Americans, the genotype distribution is in the same direction as for the 2 other groups. The T allele is associated with worse response and this is likely responsible for the strong association in the total sample. A much smaller effect size of this association in Europeans owing to differences in linkage disequilibrium structure and thus correlation with the putative functional variant might necessitate much larger samples to detect statistically significant associations in this subgroup.

Because of large differences in MAF between the ethnic subgroups, with the SNP being more common in African Americans (MAF=28%) than the 2 other ethnic groups (MAF < 6%), and because poorer treatment response for minorities has been documented in this sample,<sup>31</sup> we were concerned that population stratification could be a confounder of this association. This is not very likely the case, because in addition to independent significant associations in African American and Hispanic patients, the associations remained significant when controlling for ethnicity as well as for a genetic estimate of the proportion of African ancestry. Although we are relatively confident that our results are not a spurious finding owing to the increased proportion of African Americans or individuals with African admixture in the nonremitter group, our analyses cannot exclude more subtle effects of population stratification within the larger continental groups.

Another interesting aspect of the association of the *CRHBP* SNP rs10473984 with treatment response is its interaction with anxious depression status. In the STAR\*D sample, the qualifier anxious depression is associated with a poorer treatment response overall,<sup>31</sup> and data presented by Fava et al<sup>34</sup> suggest that it may be a valid diagnostic subtype of MDD, with distinct psychiatric comorbidities and clinical and sociodemographic features. Although rs10473984 was not associated with the presence of anxious depression itself (data not shown), we observed that for this SNP, the association with treat-

ment response was mostly carried by individuals with anxious depression (43% of all STAR\*D subjects<sup>34</sup>). Two previous articles have shown that associations of variants in *CRHR1* were also most pronounced in patients with anxious depression.<sup>21,22</sup> A series of previous findings<sup>1,40-42</sup> suggest that the CRH system is critically involved in both features of anxiety and depression, and it is thus possible that patients with anxious depression show higher activity of this system than patients with nonanxious depression. Genetic variants in genes regulating the CRH system may have a greater impact on treatment response in anxious depression.

*CRHBP* encodes the CRH-binding protein, which is present in the circulation and the interstitial spaces as a soluble 37-kDa glycoprotein that binds CRH and all urocortins with high affinity, reduces their bioavailability, and prevents their binding to CRH receptors.<sup>43-45</sup> *CRHBP* is highly expressed in a series of tissues, including the brain,<sup>46-48</sup> thereby permitting its role in modulation of CRH neurotransmission.<sup>47</sup> De Luca et al<sup>49</sup> have already reported on an association of a variant within this gene with suicidal behavior in patients with schizophrenia.

rs10473984 is located at the 3' end of *CRHBP*. At least in populations of African descent, the linkage disequilibrium structure would allow for this SNP to tag potential causal variants in the last intron and exon of the gene, which could influence splicing, change the coding sequence, or lead to differences in gene expression.<sup>50</sup> Using the HapMap gene expression and SNP genotype data,<sup>51</sup> we could not find any significant effect of this SNP on *CRHBP* messenger RNA expression in lymphoblastoid cell lines (data not shown). Regulatory effects are known to be tissue specific, so this SNP might well be associated with *CRHBP* levels in other tissues. The fact that this SNP was associated with corticotropin levels (Figure 4B) could support a functional effect of this variant. The T allele, associated with poorer response to citalopram treatment, was also associated with higher corticotropin serum concentrations in depressed and nondepressed individuals. This might suggest that this allele is associated with reduced *CRHBP* expression and thus higher levels of free CRH, thereby increasing corticotropin secretion. In addition, individuals with clinically significant depressive symptoms carrying the GG genotype (associated with best treatment outcome) of this SNP showed the least degree of dexamethasone suppression of corticotropin. Previous studies have shown that depressed patients with dexamethasone nonsuppression of HPA-axis activation at treatment initiation have a beneficial treatment-response profile.<sup>39</sup>

In conclusion, despite some limitations, such as the lack of a formal replication and incomplete genetic coverage, which was most pronounced in *UCN* and *UCN2*, our results support a role of the CRF system in treatment response to citalopram in patients with MDD and expand upon previous preclinical and clinical studies that demonstrated a central role of this system in the pathophysiology of depression and mechanism of action of antidepressants. Our results also support the notion that genetic variants in this system might be most relevant in predicting treatment response in anxious depression.

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**Author Contributions:** Dr Binder had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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In the past 3 years, Dr Owens has had research grants from National Institutes of Health, Eli Lilly and Company, Pfizer, GlaxoSmithKline, Lundbeck, Cyberonics, Ortho-McNeil Janssen, and AstraZeneca. He has served as a consultant to H. Lundbeck A/S and received speaker's honoraria from Eli Lilly and Company. Dr Owens has a patent entitled "A method to estimate transporter occupancy."

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Dr Trivedi has been a consultant for Abbott Laboratories, Akzo (Organon), AstraZeneca, Bayer, Bristol-Myers Squibb, Cephalon, Cyberonics, Eli Lilly and Company, Fabre-Kramer Pharmaceuticals, Forest Pharmaceuticals, GlaxoSmithKline, Janssen Pharmaceutica, Johnson & Johnson, Eli Lilly and Company, Meade Johnson, Neuronetics, Parke-Davis Pharmaceuticals, Pfizer, Pharmacia & Upjohn, Sepracor, Solvay Pharmaceuticals, Vantage-Point, and Wyeth-Ayerst Laboratories; has served on speak-

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Currently, Dr Nemeroff serves on the scientific advisory boards for Astra-Zeneca, Johnson & Johnson, Pharma Neuroboost, Quintiles, and National Alliance for Research on Schizophrenia and Depression. He previously served on the Forest Laboratories scientific advisory board. He has received grant money from the National Institutes of Health. He serves on the Board of Directors of AFSP, NovaDel Pharmaceuticals, Mt Cook Pharma, and the George West Mental Health Foundation. He owns equity in CeNeRx and Reevax. He owns stock or stock options in Corcept, Mt Cook Pharma, and NovaDel.

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