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Abstract

Objective—Genetic variation within the hypothalamic-pituitary-adrenal (HPA) axis has been linked to risk for depression and antidepressant response. However, these associations have yet to produce clinical gains that inform treatment decisions. We investigated whether variation within HPA axis genes predicts antidepressant outcomes within two large clinical trials.

Methods—The test sample comprised 636 patients from the iSPOT-D study who completed baseline and 8-week follow-up visits, and who had complete genotyping data. We tested the relationship between genotype at 16 candidate HPA axis SNPs and treatment outcomes for three commonly-used antidepressants (escitalopram, sertraline and venlafaxine-XR) using multivariable linear and logistic regression, with Bonferroni-corrected alpha control. Response and remission were defined using the Hamilton Depression Rating Scale (HDRS). Findings were then validated using the NIMH-sponsored PReDICT study of outcome predictors in treatment-naïve patients with MDD.

Results—We found that the rs28365143 variant within the corticotropin-releasing hormone binding protein (CRHBP) gene predicts antidepressant outcomes for remission, response and symptom change. Patients homozygous for the G allele of rs28365143 had greater remission rates, response rates and symptom reductions. These effects were specific to drug class. Patients homozygous for the G allele responded significantly better to escitalopram and sertraline (selective serotonin reuptake inhibitors) than did A allele carriers. In contrast, rs28365143 genotype was not associated with treatment outcomes for the serotonin norepinephrine reuptake inhibitor venlafaxine. When patients were stratified by race, the overall effect of genotype on treatment response remained. In the validation sample, the GG genotype was again associated with favorable antidepressant outcomes, with comparable effect-sizes.

Conclusions—These findings suggest that a specific CRHBP SNP, rs28365143, has a role in predicting which patients will improve with antidepressants, and which type of antidepressant may be most effective. The results add to the foundational knowledge we need to advance a precision approach to personalized antidepressant choices.

Introduction

Disturbances of the hypothalamic-pituitary-adrenal (HPA) axis, a major component of the mammalian response to stress, have been consistently documented in Major Depressive Disorder (MDD). Both corticotropin-releasing hormone (CRH), a key regulator of the axis, and cortisol, its end product, play critical roles in coordinating the endocrine, behavioral, autonomic, and immune responses to stress (Steckler et al., 1999). Plasma concentrations of
cortisol have been linked not only to risk for depression but also to MDD disease course and treatment outcome (Juruena et al., 2006; Hinkelmann et al., 2012). Although genetic variation within HPA axis genes (including CRH and its receptors) has been linked to depression risk and prognosis, it remains unknown whether genetic variation within the HPA axis contributes to a differential prediction of outcomes for specific types of antidepressants. In this study, we sought to determine whether individual variation within five HPA axis genes was predictive of antidepressant response, and if so, whether this prediction was related to antidepressant type.

Dysregulation of the HPA axis was one of the earliest reported biological characteristics of MDD (Carroll et al., 1976; Rubinow et al., 1984), and appears to be a central feature of the pathophysiology of depression. Depressed individuals have been shown to exhibit higher salivary and plasma cortisol concentrations (Trestman et al., 1995; Juruena et al., 2006), as well as impaired cortisol suppression by dexamethasone and altered 24-hour cortisol amplitude (Juruena et al., 2006; Posener et al., 2000). In addition, high levels of morning cortisol have been linked to increased risk for depression in adolescents (Harris et al., 2000; Goodyer et al., 2009). Unsurprisingly, upstream regulators of cortisol, including CRH and its receptors and binding protein, have also been implicated in depression. Elevated CRH concentrations have been repeatedly reported both in the CSF of depressed individuals (Nemeroff et al., 1984; Banki et al., 1987) and suicide victims (Arató et al., 1989). Disturbances in the HPA axis have also been postulated to correspond with disease course, as HPA axis dysfunction appears to worsen with the number of successive depressive episodes a patient experiences (Gervasoni et al., 2004; Hatzinger et al., 2002). Yet still other studies have reported either no difference between cortisol levels in depressed and control patients or reduced cortisol levels in depressed individuals (Piccirillo et al., 1994; Oldehinkel et al., 2001). Thus, the picture regarding HPA dysfunction, and cortisol dysfunction in particular, appears to be a mixed one, varying as a function of type of measurement, time of measurement, and patient characteristics (for review: Belvederi Murri et al., 2014).

Given the potential role of CRH and cortisol in the pathophysiology of depression, variation in the genes regulating this pathway has been explored as a potential risk factor for this disorder. CRH binds to two membrane-bound receptors (CRHR1 and CRHR2), and forms a complex with corticotropin-releasing hormone binding protein (CRHBP) both in the periphery and the central nervous system (CNS). The formation of this complex regulates the amount of free CRH, which in turn controls direct CNS response to CRH and indirectly impacts the amount of cortisol released by the adrenal glands. Cortisol then binds to the glucocorticoid receptor NR3C1 throughout the cortex and rest of the body, regulating downstream biological and behavioral responses to stress (Turner et al., 2010). Cortisol system variation that has been found to associate with depression diagnosis includes two SNPs within CRHBP (rs7728378 and rs1875999) (Claes et al., 2003), as well as one SNP in the CRHR1 gene (rs110402) which is thought to mediate the relationship between childhood trauma and the development of depression (Heim et al., 2009). Another variant in CRHR1, rs4076452, was found to correlate significantly with depression severity rating (as well as psychosis in depression) (Schatzberg et al., 2014). In addition, one CRHR2 SNP (CRHR2s183) has been associated with unipolar depression, though this association was not replicated in a separate cohort (Villafuerte et al., 2002). A constellation of SNPs within the
glucocorticoid receptor NR3C1 was also found to associate with both cortisol levels and psychosis in MDD (Schatzberg et al., 2014), providing further evidence that HPA axis plays a key role in depressive symptomatology.

HPA axis dysregulation may also be associated with recovery from depression. Treatments for MDD, including pharmacotherapy, may (at least partially) begin to normalize these HPA axis abnormalities (De Bellis et al., 1993; Kling et al., 1994; Hinkelmann et al., 2012), suggesting that the normalization process itself may be related to treatment outcome. Moreover, it is possible that baseline HPA axis function (potentially influenced by variation in genotype), may interact with pharmacotherapy to impact probability of treatment response, independent from treatment-dependent changes in HPA axis function. Recent literature appears to support this hypothesis: two loci within CRHBP (the SNP rs10473984, as well as a 3-SNP haplotype), together with one locus within CRHR2 (rs2270007), have all been associated with treatment response to various antidepressants (Licinio et al., 2004; Liu et al., 2007; Papiol et al., 2007; Binder et al., 2010). However, it is unknown whether additional variants within this system contribute to general or class-specific antidepressant response in depression. In sum, it appears that genetic variation is related to both HPA axis function and depression treatment response, but the precise interaction between genetics and treatment response has yet to be fully explored.

Our primary goal was to establish whether variation in five CRH and cortisol-related genes (CRH, CRHR1, CRHR2, CRHBP and NR3C1) contributes to symptom outcomes following acute antidepressant treatment in MDD. We first assessed whether variation in these genes predicts symptom reductions across three commonly prescribed antidepressants (escitalopram, sertraline, and venlafaxine XR), irrespective of antidepressant type. Next, we assessed whether CRH and cortisol-related genotype predicted outcomes based on antidepressant, SSRI (escitalopram and sertraline) versus SNRI (venlafaxine XR). The ultimate goal was to develop treatment-specific response probabilities based on genetic variation in order to guide treatment decisions in a personalized manner.

**Methods**

**Trial design**

The iSPOT-D is a multi-site randomized practical clinical trial designed to identify predictors of antidepressant efficacy in Major Depressive Disorder (MDD). A total of 1008 adults (ages 18-65 years old) with MDD were assessed across 17 sites in the US, Netherlands, Australia, New Zealand, and South Africa between December 2008 and January 2012 (Williams et al., 2011).

**Participant inclusion and exclusion criteria**

Participants met primary inclusion criteria including a DSM-IV diagnosis of non-psychotic MDD as assessed by the Mini International Neuropsychiatric Interview version 5.0, score of 16 or greater on the HDRS, and age of 18-65 years. Participants were excluded if they had a positive urine toxicology screen, comorbid psychotic disorder, PTSD, and/or Axis II personality disorder and any medical condition that might interfere with assessments or
medication safety. Additional details are provided in the protocol paper (Williams et al., 2011).

**Treatment**

Participants, who were either antidepressant-naive or underwent a wash-out period of at least one week, were randomized to receive escitalopram, sertraline, or venlafaxine XR using a block design by PhaseForward’s validated web-based Interactive Response Technology (Williams et al., 2011). Research personnel involved in subsequent study assessments were blind to randomization. Once randomized, medications were adjusted by treating clinicians according to clinical judgement and recommended dosage ranges. Treatment for concomitant medical conditions was permitted and recorded (for sample details, Saveanu et al., 2015).

**Genotyping**

Genotype extraction was performed using the Puregene DNA method (Quiagen, Valencia, CA) on EDTA-treated blood. Genotyping was completed using the Illumina VeraCode Golden Gate SNP genotyping platform (Illumina, Hayward, CA) by Covance, Inc. (Seattle). Sixteen candidate SNPs from four HPA axis genes, along with 663 other candidate loci in genes that have been previously implicated in depression, were selected for genotyping. To specifically investigate the role of the HPA axis in depression, the present study only focuses on the 16 HPA axis SNPs within the genes CRH, CRHBP, CRHR1, CRHR2, and NR3C1.

We also screened for deviation from Hardy-Weinberg equilibrium at each of the 16 SNPs of interest (Table 1). Because our sample included Caucasian and minority groups, recorded using standard World Health Organization formats, we also calculated the p-values for deviation from Hardy-Weinberg Equilibrium for the two largest racial subgroups in our sample, white and black participants. Although 3 SNPs deviated from Hardy-Weinberg equilibrium in the entire sample, none deviated significantly in either ethnic subgroup.

**Characteristics of the sample with complete genotyping data**

Of the 1008 patients randomized to antidepressant treatment, 900 had complete genotype data. Of these, 636 also completed the 8-week post-treatment follow-up visit. The 636 patients who were genotyped (i.e. had genotype information at >50% of the candidate SNPs) and completed 8 weeks of treatment were used for the present analysis (for details, Supplementary CONSORT Figure 1).

As shown in Table 2 the 636 patients with complete genotype data did not differ in demographic, clinical and medication dose characteristics from the initially randomized sample of 900 patients with complete genotype information or from the full randomized sample of 1008. Allelic frequency of all 16 SNPs also did not differ between completers and the full sample (Supplementary Table 1).

**Treatment Outcome Measures**

The primary outcome measures used in this study were consistent with the protocol for the iSPOT-D study (Williams et al., 2011). Acute outcomes were assessed after 8 weeks of
clinician-monitored antidepressant treatment using the clinician-rated symptom change assessed by the 17-item Hamilton Depression Rating Scale (HDRS). In the present study, we assessed percent reduction in HDRS score, absolute score reduction, response (defined as a ≥50% reduction in HDRS-rated symptoms) and remission (defined as a score of ≤7 on the HDRS).

**Statistical Analysis: Treatment Outcome Prediction by Genotype**

The first aim of the present study was to determine whether genotype of any of the 16 candidate SNPs predicted magnitude of symptom improvement after 8-weeks of antidepressant treatment. Linear regression for each SNP was performed with percent HDRS score reduction as the primary outcome measure and the candidate SNP in Table 1 as a predictor. We followed an additive allelic model, with genotype coded as 0, 1, or 2, representing number of alternate alleles in each individual. However, to preserve power in SNPs at which fewer than 20 participants had the homozygous alternate genotype, genotype was coded as a binary variable, representing the presence of any alternate alleles. Participant age and recruitment site were included as covariates in each regression model, because they were significantly associated with our outcomes of interest as has been previously reported (Schatzberg et al., 2015). As in Schatzberg et al. (2015), recruitment sites were grouped by geographic location into seven groups, each with a minimum of 50 participants. Each SNP was then individually regressed on outcome in a regression model including covariates, and all SNPs that significantly predicted outcome above and beyond covariates are reported here. We used the eigenvalue-based method described in Li and Ji (2005) to determine the effective number of hypotheses being tested given observed linkage disequilibrium between candidate SNPs; this yielded 13 effective hypotheses and a corrected p-value threshold of < 0.003938. For the purposes of completeness, we report all SNPs with uncorrected p-values below 0.05.

Linear regression was used to assess prediction of continuous measures of symptom change. Because there are small statistical differences between using percent and linear reduction as an outcome, all results obtained with percent change as a the treatment outcome variable were validated using absolute HDRS score reduction controlling for initial score as well.

Following this same approach, we used logistic regression to assess binary treatment outcomes of response and remission defined above. Age was included a covariate for these models given associations with outcomes, and baseline HDRS score was also included for remission status. A corrected p-value of .00398 was used as the threshold for statistical significance, with all p-values of < 0.05 reported for completeness. This correction was based on adjustment for the number of SNPs (each was a separate hypothesis) but not for outcome measures which are metrics derived from the same measure.

**Validation sample**

The SNPs that were significantly associated with treatment response in the initial iSPOT-D cohort were then validated using an independent sample of patients from the Predictors of Remission in Depression to Individual and Combined Treatments (PReDICT) trial (Dunlop et al., 2012). To achieve equivalence of treatment arms in the original iSPOT-D and
validation PReDICT samples, only PReDICT patients assigned to either of the two pharmacotherapy arms, consisting of treatment with either escitalopram or duloxetine, were included for validation. Although duloxetine was not used in the original iSPOT-D cohort, it belongs to the same medication class (SNRI) as venlafaxine. The full description of this sample, including inclusion and exclusion criteria, genotyping, quality control, and sample characteristics have been published previously (Dunlop et al., 2017). Further details can be found in the Supplementary Methods. In this sample, rs28365143 genotype was imputed based on genome-wide markers from the Illumina Omni-Express array data using the 1000 Genomes Project Phase 3 Reference haplotypes with IMPUTE2 (Howie et al., 2009) and pre-phased with SHAPEIT2 (Delaneau et al., 2012). Among participants with complete imputed genotypes for rs28365143 (N = 141), the best-guess genotype call rate in the full sample was 0.9338.

Validation was evaluated using the primary outcome of percent reduction in HDRS score and the secondary outcomes of absolute HDRS reduction, response, and remission. Due to the smaller sample size of the validation cohort, particularly the limited number of participants with the A allele at rs28365143, and the focus on two rather than three medication arms, we focused the validation on the overall effect of SNP on treatment response and not on additional interactions testing differential outcomes based on antidepressant class.

**Statistical Analysis: Treatment Outcome Prediction by Genotype and Treatment Class**

To determine whether the relationship between genotype and treatment outcome was moderated by antidepressant type, treatment arm was introduced as an interaction term to the logistic and linear models mentioned above meeting the uncorrected significance threshold of p < 0.05 in the original sample. The significance of the interaction between drug type (SSRI vs. SNRI) and genotype was reported, along with the main effect for genotype within each medication class. To verify that any significant differences in the SSRI class were not due to increased sample size of that class, we also conducted regressions to determine the interaction between individual drug (escitalopram, sertraline, or venlafaxine) and genotype. The interaction term between genotype and each drug type was then assessed for significance (p < 0.05).

As in the previous analysis, the above multivariable linear regressions were conducted with percent change as the primary outcome variable, followed by multivariable logistic regressions with response and remission as the binary dependent outcome variables. The same set of covariates (age, site, and initial score for reduction and remission) were used as in the preceding main effect analysis. This interaction was only explored in the original iSPOT-D cohort due to power considerations and the smaller sample size of the validation (PReDICT) sample.

**Statistical Analysis: Stratification by Ethnicity**

Given the potential for population stratification to cause false positive associations in genetic association studies, we stratified our original (iSPOT-D) sample by Caucasian vs. non-Caucasian participants and examined the association between genotype and outcome for
each SNP significantly associated with treatment outcome in the entire sample. More specific stratification by ethnicity was not possible given the small number of non-Caucasian participants in our sample belonging to each ethnic subgroup (total of 254 participants; 97 Black, 89 Other, 43 Asian). Due to power considerations and the small number of patients with many of the alternate genotypes receiving SNRI treatment, stratifying by ethnicity, genotype, and treatment type was not performed. In addition, due to the smaller size of the validation sample (N\textsubscript{Caucasians}=67, N\textsubscript{Others}=74), stratification by ethnicity was only performed in the original and larger iSPOT-D cohort.

### Results

#### Treatment Outcome Prediction by Genotype

Of the 16 SNPs, only one (rs28365143, within the gene CRHBP) significantly predicted the primary outcome measure (percent HDRS score reduction) before (p < 0.05) and after Bonferroni correction (p < 0.003938). Overall, the GG genotype (homozygous reference) was associated with a larger percent reduction in HDRS score compared with genotypes containing A alleles (AG/AA) (β = -0.12, p = 9.9 × 10\textsuperscript{-5}; Figure 1) (for results of candidate SNPs not meeting statistical significance see Supplementary Table 2). Similarly, only rs28365143 emerged as a significant predictor of absolute HDRS score reduction, with the GG genotype again associated with greater score reductions (β = -2.51, p = 2.5 × 10\textsuperscript{-4}).

The same associations were observed for the two clinical outcome measures, response and remission, with significant associations between rs28365143 genotype and response (OR = 0.46, uncorrected p = 0.0017) and remission (OR = 0.36, uncorrected p = 1.6 × 10\textsuperscript{-4}). Again, rs28365143 was the only SNP that had a significant association with outcome, and these associations withstood Bonferroni correction (Figure 1).

#### Validation sample

In the validation sample, participants who were homozygous for the reference allele (“GG” genotype) also showed greater percent reductions in HDRS score after 8 weeks of pharmacotherapy with either escitalopram or duloxetine (β = -0.178, p = 0.034). When it came to the secondary outcomes of interest – absolute reduction, response, and remission – the homozygous reference genotype was similarly associated with a trend toward a comparably favorable effect on antidepressant outcomes. These results for the secondary outcomes did not reach the threshold for statistical significance, likely due to the smaller size of the validation sample. Importantly, however, the size of the effect of the rs28365143 genotype on treatment outcome was equivalent between the original iSPOT-D sample and the validation PReDICT sample (Table 3). Arguably, the effect size is a more comparable metric for establishing reproducibility than p value.

#### Treatment Outcome Prediction by Genotype and Treatment Class

There was additionally a significant interaction between rs28365143 genotype and type of antidepressant (p = 0.014) in the original iSPOT-D cohort when predicting percent reduction in HDRS score. Patients homozygous for the reference allele (“GG”) had better outcomes (greater percent symptom reductions) than carriers of the alternate “A” allele when treated
with an SSRI (escitalopram and sertraline arms combined, $\beta_{\text{SNP}} = -0.17$, $p = 5.02 \times 10^{-6}$). This result held when the escitalopram and sertraline arms were analyzed individually (Supplementary Table 3). While rs28365143 genotype was not a significant predictor of treatment outcome in patients taking the SNRI venlafaxine ($\beta_{\text{SNP}} = -0.01$, $p = 0.89$), the homozygous reference genotype was linked to a 16-18% greater reduction in symptoms for patients taking SSRIs (Escitalopram: $\beta_{\text{SNP}} = -0.18$, $p = 5.81 \times 10^{-4}$, Sertraline: $\beta_{\text{SNP}} = -0.16$, $p = 0.0020$). The interaction of rs28365143 and type of antidepressant was reproduced when we analyzed absolute HDRS reduction ($\beta = -3.21$, $p = 0.031$) and response ($\beta = -1.48$, $p = 0.0066$). When it came to remission, while the effect of A allele was significant within both SSRIs combined ($\beta_{\text{SNP}} = -1.33$, $p = 4.47 \times 10^{-5}$) and each individually (Escitalopram: $\beta_{\text{SNP}} = -1.30$, $p = 0.0032$, Sertraline: $\beta_{\text{SNP}} = -1.38$, $p = 0.0036$), the overall interaction was not significant ($\beta = -1.03$, $p = 0.071$). As illustrated in Figure 2, patients homozygous for the reference allele ("GG") had consistently better outcomes than carriers of the alternate "A" allele, irrespective of how outcome was quantified, and specifically when treated with SSRIs. Again, these interactions were not assessed in the validation cohort due to its limited sample size.

**Stratification by Ethnicity**

In both Caucasian and non-Caucasian subgroups of the original iSPOT-D cohort, the alternate allele of rs28365143 was again associated with smaller percent reductions in HDRS score (Caucasian: $\beta = -0.11$, $p = 0.009$; non-Caucasian: $\beta = -0.12$, $p = 0.011$), and the magnitude of this association was comparable to that of the entire sample (Supplementary Table 4). Due to the reduction in power due to stratification, however, the significance of this association did not withstand multiple hypothesis testing correction in either subgroup. Similarly, the magnitude and direction of the association between genotype and treatment outcome was comparable to that in the entire sample when examining the secondary outcomes of linear reduction, response, and remission.

**Discussion**

We report on new evidence for the role of the SNP rs28365143, within an intron of the CRHBP gene, in predicting antidepressant treatment outcomes. This predictive relationship was specific to SSRIs and not the SNRI venlafaxine-XR, particularly when looking at Caucasian participants. Patients homozygous for the reference “G” allele showed greater symptom improvement than patients who were carriers of the alternate “A” allele, particularly in response to escitalopram and sertraline. These SNP-outcome relationships were reproducible across multiple measures of symptom improvement within the original sample, and the association with the primary outcome of percent reduction was replicated in a separate validation cohort.

To our knowledge, the present findings are the first to suggest that rs28365143 has a specific and robust role in predicting antidepressant outcomes for multiple SSRIs. The effects for rs28365143 survived stringent correction for multiple hypothesis testing in the full sample but not in the Caucasian subgroup, although the magnitude of the association was the same. Given the strength of this association and the lack of association between the 15 other HPA-
axis SNPs evaluated in the present study, these data indicate that rs28365143 may have a specific role in SSRI outcomes. This suggestion is consistent with a previous report of an association between rs28365143 and self-reported symptom improvement (Binder et al., 2010), although this previous observation did not withstand correction for multiple testing, and did not extend to response and remission rates.

It is notable that the present findings show that rs28365143 is a predictor of remission, given that remission is the pathway to recovery from major depression (Nierenberg et al, 2001). While the allele frequency of the minor “A” allele was relatively low in both the original and validation samples (7.4 and 7.9% respectively), it is important to consider that the major allele, comprising 92.6 and 92.1% of each sample, was associated with greater probability of remission. The ability to identify those more likely to remit on an antidepressant, in addition to identifying those not likely to, would be an important improvement over current heuristic practices. The observed odds ratios for prediction of remission (OR = 0.36) are large enough to illustrate that the findings have potential utility for clinical translation. To explicitly test for clinical translational utility, it would be important to undertake a randomized controlled trial that tests the additional benefit of prospectively-guided treatment based on this candidate SNP versus traditional heuristic prescribing. Investigating the clinical impact of using genetic information to guide antidepressant treatment choices, as well as the sensitivity and specificity of genetic markers and other biomarkers at identifying responders and non-responders, is a line of inquiry that has been advocated by many others in the field (Singh et al., 2014; Bousman et al., 2017; Williams et al., 2016; Rosenblat et al., 2017).

Given the fact that epistatic interactions may interact with different background allele frequencies in different ethnicities, it may be important to consider these results in the context of stratification by ethnicity. Unfortunately, the small number of non-Caucasian participants in the present study made stratification by non-Caucasian ethnicity impractical. While the impact of SNP on outcomes observed in Caucasian and non-Caucasian subgroups was of the same magnitude and direction as the effect that was seen in the overall sample, the majority of these associations did not survive correction for multiple hypothesis testing (excluding remission in the Caucasian sample). Further, well-powered studies are necessary to verify this association between rs28365143 on depression outcomes as well as the interactions with drug class in a variety of different ethnic backgrounds.

Because CRH has been implicated broadly in depression and its treatment, we had anticipated predictive relationships among the 15 other HPA axis SNPs and treatment outcomes. It is possible that treatment-predictive relationships for the other HPA axis SNPs depend also on moderation by other demographic or patient characteristics or by different antidepressant mechanisms of action. In the STAR*D trial, rs10473984 (also within CRHBP) was associated with decreased responsiveness to escitalopram (Binder et al., 2010). However, we note that this association was specific to African-American and Hispanic subgroups of the STAR*D sample, while the present study was primarily Caucasian. In both Mexican-Americans and Han Chinese, three haplotype-defining SNPs (rs1876828, rs242939 and rs242941) have been related to response to desipramine and fluoxetine (Licinio et al., 2004; Liu et al., 2007). We did not find such relationships for these three SNPs and response to escitalopram and sertraline. It is of note, however, that both previous studies only
observed this relationship in the high-anxiety subgroup of their samples, consisting of 54 participants in Licinio et al. (2004) and 85 participants in Liu et al. (2007). The small samples in which this association was observed and the different ethnic background of each of these samples (compared to our predominantly Caucasian sample) makes the lack of replication less surprising. Fluoxetine also belongs to the general SSRI class of antidepressants, yet has a distinct profile of pharmacologic specificity and tolerability. Given this distinct profile, it is possible (although speculative) that distinct CRH SNPs have a specific impact on response to fluoxetine that is separate from the effect of rs28365143 on escitalopram and sertraline. This possibility may apply more obviously to desipramine, which belongs to the tricyclic (and not the SSRI) class of antidepressant.

The precise biological role of rs28365143 also warrants further investigation. It is located within an intron of CRHBP, and its function, if any, remains largely unexplored. CRHBP is thought to regulate levels of free CRH available for receptor binding, which in turn affects downstream cortisol release (Behan et al., 1997; for a more complete discussion of CRHBP and its roles, see Extended Discussion in Supplemental Materials). In addition, CRH also has direct effects within the CNS governing response to stress, particularly in the amygdala and locus coeruleus (Lee and Davis et al., 1997; Shepard et al., 2000; Jedema & Grace et al., 2004). Thus, it is possible that different alleles at this locus or nearby may alter expression, structure, or function of CRHBP, thus impacting either downstream effects of CRH and cortisol, direct response to stress, or both. When it comes to identifying the causative allele, it is possible that the true causative SNP may be in linkage disequilibrium with rs28365143. A SNP Annotation and Proxy Search (SNAP) reveals 21 nearby SNPs whose correlation with rs28365143 have an $r^2$ value of > 0.7 (Johnson et al., 2008). Although none of these are eQTLs, two of the proxy SNPs (rs41272246 and rs75439203) are classified as “likely to affect transcription factor binding” by RegulomeDB (Boyle et al., 2012). It is possible that variants of CRHBP may be affected differentially by changes in serotonergic and noradrenergic neurotransmission caused by pharmacotherapy for depression. A shift in the balance between the actions of these neurotransmitters and the CNS effects of CRH (in addition to downstream production of cortisol in response to stress caused by differential levels of CRH) may account for the differential treatment response associated with rs28365143 genotype. Further work is required to clarify the causal SNP responsible for this association with treatment response and to clarify the potential biological mechanism for this association, perhaps starting with the effect of rs28365143 on cortisol levels and reactivity.

It is also worth considering the clinical relevance of our finding that the genotype of CRHBP SNP rs28365143 may predict differential response to the SSRIs escitalopram and sertraline, but not necessarily venlafaxine, in the iSPOT-D cohort. While duloxetine belongs to the same class of medication as venlafaxine (both are SNRIs), the PReDICT validation sample was not large enough to test this differential response according to treatment class. Mechanistically, the precise effects of antidepressants at the neurotransmitter level, and the role of cortisol and genetic variation in moderating these effects, remains speculative. While cortisol-moderating genes within the HPA axis may exert their treatment-modifying effects via serotonin and norepinephrine, it is also possible that this effect involves the HPA axis as well (O’Hara et al., 2007; Nicholson et al., 2014). For example, Binder et al. found that an
allele of another SNP within CRHBP was shown to associate with not only lack of response to escitalopram in MDD, but also exaggerated dexamethasone suppression of cortisol and higher plasma corticotropin levels (Binder et al., 2010). Overall, more research is necessary to determine whether CRHBP rs28365143 influences CRH or cortisol levels, and to clarify the exact ways in which cortisol, norepinephrine, and serotonin levels interact and are influenced by pharmacologic therapy for MDD. It may be possible in the future to combine genetic information with information about brain function and environmental factors to further refine treatment decisions (Goldstein-Piekarski et al. (2016)).

There are several important limitations to the present study. First, the sample was predominantly Caucasian. We addressed this issue to the greatest extent possible by implementing analyses with a stratification of Caucasian vs. non-Caucasian participants, which did not affect the magnitude or direction of the association. The findings also replicated in the PReDICT sample, which was more heterogeneous. Seeking to replicate the current results in distinct and homogenous ethnic and racial subgroups would be an important next step. In addition, future studies could directly examine the prediction of remission in patients stratified by both rs28365143 genotype and treatment type, to examine the drug class-specific nature of this effect and determine whether it is altered by differences in genetic background. The focus on a sample of completers rather than on an intent-to-treat sample may be an additional limitation, as this could have introduced bias by excluding participants who did not complete the 8-week follow-up visit. This choice was made in order to relate candidate genetic variants to treatment outcomes, consistent with a biomarker trial approach. Importantly, HPA axis genotype had no effect on study completion in any of 15 candidate SNPs. In addition, other baseline characteristics such as age, sex, race, initial depression severity, and age of onset did not differ between the two groups.

In conclusion, this work supports a potential role for the SNP rs28365143 within CRHBP in predicting response to pharmacotherapy for depression. To our knowledge, this is the first report on a significant role of this SNP in prediction of treatment outcomes in MDD, and also the first study to compare the effects of HPA axis variation across treatment types. Further work is necessary to understand whether the effect of this SNP pertains to other therapies such as electroconvulsive therapy or cognitive behavior therapy for MDD.

Overall, pharmacogenomics appears to be a promising avenue for the personalization of psychiatric pharmacotherapy, specifically through targeted genotyping. We are optimistic that this precision approach to antidepressant choices will eventually help improve physicians' treatment decisions, decrease the number of failed treatment trials and time to remission, and limit disability due to MDD.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

**Acknowledgments**

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medications, escitalopram and duloxetine, respectively, and were otherwise uninvolved in study design, data collection, data analysis, or interpretation of findings.

References


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Turner JD, Alt SR, Cao L, Vernocchi S, Trifonova S, Battello N, Muller CP. Transcriptional control of the glucocorticoid receptor: Cpg islands, epigenetics and more. Biochem Pharmacol. 2010; doi: 10.1016/j.bcp.2010.06.037


Figure 1. CRHBP rs28365143 genotype and predicted reductions in depressive symptomatology based on regression models in both the original and validation cohorts.

Predicted percent symptom reduction, as well as probability of response and remission, were calculated for each CRHBP rs28365143 genotype using the regression models described above. To calculate the expected output for an “average” participant, all other covariates were assigned to the mean value for that variable in the cohort. P-values reflect the p-value of the β coefficient in the linear or logistic regression model.

** indicates p < 0.003938, threshold Bonferroni-corrected for 13 hypotheses

* indicates p < 0.05, uncorrected
Figure 2. Drug class-specific effects of CRHBp rs28365143 genotype on response to pharmacotherapy in the original iSPOT-D cohort
The probabilities above represent the percent HDRS score change, and probability of response and remission given the overall regression model with an interaction term between drug (coded as SSRI vs. SNRI) and genotype. As in Figure 1, all other covariates (age, sex, initial score, site) were set to the mean levels of the entire cohort to isolate the effect of genotype.
* indicates p-value < 0.05
**Table 1**

Candidate SNP information. Positional information was determined using genome build GRCh37.p7, allele frequency according to 1000 Genomes Project (Ensembl). When multiple locations and/or nucleotides were listed for a given SNP, the forward strand location and genotype was selected.

<table>
<thead>
<tr>
<th>SNP ID</th>
<th>Gene</th>
<th>Chr:pos</th>
<th>Major / minor</th>
<th>Minor allele frequency (%)</th>
<th>Hardy-Weinberg p-value</th>
<th>H-W p-value: White only</th>
<th>H-W p-value: Black only</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs3176921</td>
<td>CRH</td>
<td>8:66179144</td>
<td>A/G</td>
<td>17.2</td>
<td>2.2 × 10^{-8}</td>
<td>0.40</td>
<td>0.06</td>
</tr>
<tr>
<td>rs5030875</td>
<td>CRH</td>
<td>8:66181831</td>
<td>A/C</td>
<td>5.3</td>
<td>0.95</td>
<td>0.69</td>
<td>0.76</td>
</tr>
<tr>
<td>rs10055255</td>
<td>CRHBP</td>
<td>5:76968168</td>
<td>T/A</td>
<td>46.7</td>
<td>0.0013</td>
<td>0.26</td>
<td>0.26</td>
</tr>
<tr>
<td>rs28365143</td>
<td>CRHBP</td>
<td>5:76952261</td>
<td>G/A</td>
<td>7.4</td>
<td>0.36</td>
<td>0.40</td>
<td>0.40</td>
</tr>
<tr>
<td>rs110402</td>
<td>CRHR1</td>
<td>17:45802681</td>
<td>C/T</td>
<td>46.3</td>
<td>0.19</td>
<td>0.61</td>
<td>0.61</td>
</tr>
<tr>
<td>rs1876828</td>
<td>CRHR1</td>
<td>17:45834159</td>
<td>C/T</td>
<td>18.5</td>
<td>0.13</td>
<td>0.96</td>
<td>0.96</td>
</tr>
<tr>
<td>rs242924</td>
<td>CRHR1</td>
<td>17:45808001</td>
<td>T/G</td>
<td>46.1</td>
<td>0.05</td>
<td>0.40</td>
<td>0.56</td>
</tr>
<tr>
<td>rs242939</td>
<td>CRHR1</td>
<td>17:45818213</td>
<td>T/C</td>
<td>9.7</td>
<td>0.41</td>
<td>0.21</td>
<td>0.73</td>
</tr>
<tr>
<td>rs4076452</td>
<td>CRHR1</td>
<td>17:45778528</td>
<td>G/C</td>
<td>16.0</td>
<td>0.96</td>
<td>0.93</td>
<td>0.71</td>
</tr>
<tr>
<td>rs6472257</td>
<td>CRHR1</td>
<td>8:66179945</td>
<td>C/T/G</td>
<td>11.9 (G)</td>
<td>0.5</td>
<td>0.60</td>
<td>0.91</td>
</tr>
<tr>
<td>rs2267712</td>
<td>CRHR2</td>
<td>7:30672618</td>
<td>C/A</td>
<td>17.2</td>
<td>0.46</td>
<td>0.95</td>
<td>0.57</td>
</tr>
<tr>
<td>rs2270007</td>
<td>CRHR2</td>
<td>7:30660356</td>
<td>C/G</td>
<td>17.9</td>
<td>0.031</td>
<td>0.52</td>
<td>0.81</td>
</tr>
<tr>
<td>rs2284216</td>
<td>CRHR2</td>
<td>7:30672345</td>
<td>G/T</td>
<td>11.6</td>
<td>0.5</td>
<td>0.93</td>
<td>0.64</td>
</tr>
<tr>
<td>rs4723003</td>
<td>CRHR2</td>
<td>7:30686125</td>
<td>C/T</td>
<td>10.7</td>
<td>0.58</td>
<td>0.81</td>
<td>0.39</td>
</tr>
<tr>
<td>rs6918</td>
<td>NR3C1</td>
<td>5:143278056</td>
<td>A/C</td>
<td>13.8</td>
<td>13.8</td>
<td>0.17</td>
<td>0.59</td>
</tr>
<tr>
<td>rs2963156</td>
<td>NR3C1</td>
<td>5:143378931</td>
<td>C/T</td>
<td>19.2</td>
<td>19.2</td>
<td>0.94</td>
<td>0.92</td>
</tr>
</tbody>
</table>

*In these instances, because there were fewer than 20 participants homozygous for the alternate allele in our sample, regression models were based on a binary genotype classification: 0 = homozygous reference, 1 = presence of alternate allele.*
### Table 2

**Participant characteristics of completers compared with full genotyped sample**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Completers (N = 636)</th>
<th>All genotyped (N = 900)</th>
<th>p-value&lt;sup&gt;f&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>359</td>
<td>56.4%</td>
<td>508</td>
</tr>
<tr>
<td><strong>Race</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>382</td>
<td>60.1%</td>
<td>423</td>
</tr>
<tr>
<td>Black</td>
<td>97</td>
<td>15.2%</td>
<td>104</td>
</tr>
<tr>
<td>Other</td>
<td>89</td>
<td>14.0%</td>
<td>104</td>
</tr>
<tr>
<td>Asian</td>
<td>43</td>
<td>6.7%</td>
<td>51</td>
</tr>
<tr>
<td>Missing</td>
<td>25</td>
<td>3.9%</td>
<td>217</td>
</tr>
<tr>
<td><strong>Drug</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Escitalopram</td>
<td>206</td>
<td>32.4%</td>
<td>299</td>
</tr>
<tr>
<td>Sertraline</td>
<td>225</td>
<td>35.4%</td>
<td>304</td>
</tr>
<tr>
<td>Venlafaxine-XR</td>
<td>205</td>
<td>32.2%</td>
<td>297</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>mean</th>
<th>s.d.</th>
<th>mean</th>
<th>s.d.</th>
<th>p-value&lt;sup&gt;f&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of baseline visit</td>
<td>38.9</td>
<td>12.8</td>
<td>38.1</td>
<td>12.7</td>
<td>0.22</td>
</tr>
<tr>
<td>Age of Onset (years)</td>
<td>23.3</td>
<td>12.2</td>
<td>23.1</td>
<td>12.1</td>
<td>0.80</td>
</tr>
<tr>
<td>Duration of Illness (years)</td>
<td>15.1</td>
<td>12.6</td>
<td>14.4</td>
<td>12.2</td>
<td>0.30</td>
</tr>
<tr>
<td>Drug dose (mg/day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Escitalopram</td>
<td>12.0</td>
<td>6.0</td>
<td>12.1</td>
<td>7.0</td>
<td>0.83</td>
</tr>
<tr>
<td>Sertraline</td>
<td>61.0</td>
<td>33.2</td>
<td>59.8</td>
<td>33.1</td>
<td>0.69</td>
</tr>
<tr>
<td>Venlafaxine-XR</td>
<td>80.7</td>
<td>41.5</td>
<td>78.9</td>
<td>42.0</td>
<td>0.66</td>
</tr>
<tr>
<td>Equivalent dose&lt;sup&gt;II&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Escitalopram</td>
<td>89.6</td>
<td>44.9</td>
<td>90.6</td>
<td>52.4</td>
<td>0.83</td>
</tr>
<tr>
<td>Sertraline</td>
<td>91.6</td>
<td>49.7</td>
<td>89.7</td>
<td>49.6</td>
<td>0.69</td>
</tr>
<tr>
<td>Venlafaxine-XR</td>
<td>80.7</td>
<td>41.5</td>
<td>78.9</td>
<td>42.0</td>
<td>0.66</td>
</tr>
<tr>
<td>Initial HDRS score / 42</td>
<td>21.8</td>
<td>4.07</td>
<td>21.84</td>
<td>4.1</td>
<td>0.71</td>
</tr>
<tr>
<td>Post-treatment HDRS score / 42</td>
<td>9.7</td>
<td>6.36</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

<sup>p-value represents chi-sq or t-test on non-missing data only in each sample</sup>
Equivalent dosage was calculated by converting escitalopram and sertraline dosages into equivalent doses of venlafaxine XR using the formula: 

\[ 7.5 \times \text{escitalopram dose} = 1.5 \times \text{sertraline dose} = 1 \times \text{venlafaxine dose} \]

(Ogle and Akkerman et al., 2013)
Table 3
Regression coefficients for the association of SNP with treatment response in the original iSPOT-D test sample and in the PReDICT validation sample

<table>
<thead>
<tr>
<th></th>
<th>Original Sample (iSPOT-D) n = 636</th>
<th>Validation Sample (PReDICT) n = 141</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate</td>
<td>p-value</td>
</tr>
<tr>
<td>% Reduction</td>
<td>β = -0.12</td>
<td>9.9 × 10⁻⁵</td>
</tr>
<tr>
<td>Linear Reduction</td>
<td>β = -2.51</td>
<td>2.5 × 10⁻⁴</td>
</tr>
<tr>
<td>Response</td>
<td>OR = 0.46</td>
<td>0.0017</td>
</tr>
<tr>
<td>Remission</td>
<td>OR = 0.36</td>
<td>1.6 × 10⁻⁴</td>
</tr>
</tbody>
</table>