Two HPA axis genes, *CRHBP* and *FKBP5*, interact with childhood trauma to increase the risk for suicidal behavior

Alec Roy[a,*], **Colin A. Hodgkinson**[b], **Vincenzo DeLuca**[c], **David Goldman**[b], and **Mary-Anne Enoch**[b]

[a]Psychiatry Service, Department of Veterans Affairs, New Jersey VA Health Care System, East Orange, NJ, USA

[b]Laboratory of Neurogenetics, National Institute on Alcohol Abuse and Alcoholism, NIH, Bethesda, MD, USA

[c]Centre for Addiction and Mental Health, Toronto, Ontario, Canada

Abstract

Childhood trauma is associated with hypothalamic–pituitary–adrenal (HPA) axis dysregulation. Both factors increase risk for suicidal behavior. Corticotropin releasing hormone (CRH) regulates the HPA axis and its actions are moderated by a high-affinity binding protein (CRHBP). We hypothesized that *CRHBP* variation and interaction with childhood trauma might influence suicidal behavior. Moreover, there might be an additive effect with *FKPB5*, another HPA axis gene previously associated with suicidality in this dataset. African Americans were recruited: 398 treatment seeking patients with substance dependence (90% men; 120 suicide attempters) and 432 non-substance dependent individuals (40% men; 21 suicide attempters). A total of 474 participants (112 suicide attempters) completed the Childhood Trauma Questionnaire (CTQ). Haplotype-tagging SNPs were genotyped across *CRHBP* and, for completeness, across *CRH, CRHR1* and *CRHR2. FKBP5* genotypes were available. Three *CRHBP* SNPs rs6453267, rs7728378 and rs10474485 showed a nominally significant interaction with the continuous CTQ score to predict suicide attempt; rs7728378 remained significant after FDR correction. There was an additive effect with *FKBP5*: in the group exposed to high trauma, the prevalence of suicide attempt was 0.35–0.30 in carriers of either the *FKBP5* rs3800373 major homozygote or the *CRHBP* rs7728378 major homozygote and 0.58 in carriers of both major homozygotes. Individuals without either major homozygote were resilient to the effects of childhood trauma (suicide attempt prevalence 0.24). Main effects of *CRHBP* rs6453267 and *CRHR1* rs9900679, both unique to African ancestry, were detected. *CRHBP* variation may predispose, independently and additively, to suicidal behavior in individuals who have experienced childhood trauma.

[*]Corresponding author. Psychiatry Service (116A), New Jersey Health Care System, 385, Tremont Avenue, East Orange, NJ 07018-1095, USA. alec.roy@va.gov (A. Roy).

Contributors

Dr Roy designed the study, wrote the protocol, collected the patients, and participated in the writing of the paper.

Dr Enoch carried out the data analyses and participated in the writing of the paper.

Dr Goldman supervised the genetic analyses.

Dr DeLuca carried out data analysis.

Dr Hodgkinson was involved in the genetic analysis.

Appendix. Supplementary material

Disclosure/conflict of interest

None of the authors have any disclosures or conflicts of interest to declare.

Keywords

African Americans; Substance dependence; CTQ; CRH; CRHR1; CRHR2

1. Introduction

Short term activation of the stress-response system, a major element of which is the hypothalamic–pituitary–adrenal (HPA) axis, is essential for survival. In response to acute stress, hypothalamic corticotropin releasing hormone (CRH) stimulates the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary which in turn stimulates the release of the glucocorticoid hormone cortisol from the adrenal cortex. Cortisol binding to the glucocorticoid receptor (GR) and the translocation of the GR from the cytoplasm into the nucleus in order to facilitate transcription of stress-response genes is moderated by a large molecular complex that includes FKBP5, a co-chaperone of hsp90 (Binder, 2009). The availability of CRH is regulated in part by a high-affinity binding protein (CRHBP) that is widely distributed throughout the body (Westphal and Seasholtz, 2006). A large proportion of total CRH is complexed with CRHBP and is therefore unavailable for receptor (CRHR1, CRHR2) activation (Behan et al., 1997).

Long term dysregulation of the HPA axis has been noted in several stress-related psychiatric disorders. Moreover, many studies have shown that childhood trauma can result in HPA axis dysregulation in adulthood and long-lasting increased risk for psychopathology (Enoch, 2011; Mann and Currier, 2010). Numerous studies have reported HPA axis dysfunction in individuals attempting or committing suicide (Arató et al., 1989; Coryell and Schlesser, 2001; Coryell et al., 2006; Jokinen et al., 2007; Jokinen and Nordstrom, 2009; Lindqvist et al., 2008a,b; Mann and Currier, 2007; Nemeroff et al., 1988; Pfennig et al., 2005; Roy, 1992; Roy et al., 1986).

Recently, *CRHBP* has gained prominence as a candidate gene for stress-related disorders (Binder et al., 2010; De Luca et al., 2010; Enoch et al., 2008; Kertes et al., 2010; Ray, 2011). Three of these studies (Binder et al., 2010; De Luca et al., 2010; Kertes et al., 2010) also investigated *CRH, CRHR1* and *CRHR2* SNPs but found significant results only for *CRHBP*. Our study participants were African Americans, many of whom had been diagnosed with substance dependence, a known risk factor for suicidal behavior (Roy, 2001, 2002, 2003; Roy and Janal, 2007). We previously showed that childhood trauma predicted suicidality in this sample (Roy et al., 2010). Based upon the results of the studies described above, we hypothesized that *CRHBP* variation and also the interaction with childhood trauma might influence suicidal behavior. We further hypothesized that we would find phenotype associations with distal *CRHBP* SNPs since all five of the earlier studies found associations only within this region of the gene. For the sake of completeness we also analyzed the effects of variation in *CRH, CRHR1* and *CRHR2*, but expected negative results based on the earlier findings.

We previously showed that the interaction between childhood trauma and *FKBP5* variation influenced suicide attempts in this sample of African Americans (Roy et al., 2010). Since the effectiveness of CRH in stimulating ACTH with resultant cortisol release is governed in part by CRHBP and since the effectiveness of cortisol in influencing gene expression via the GR is moderated by a molecular complex that includes FKBP5, we further hypothesized that there would be an additive effect between *CRHBP* and *FKBP5* variation in the interaction with childhood trauma to predict risk for suicide attempt.

2. Methods

2.1. Participants

The sample consisted of two subsamples of African–American participants recruited from the same geographic area that were pooled for this study. The first subsample consisted of 706 African–American patients (634 men, 72 women) recruited from the Substance Abuse Treatment Program (SATP) at the Department of Veteran Affairs New Jersey Healthcare System (VANJHCS), East Orange Campus. Nearly all were inpatients however a few were recruited from the SATP outpatient clinic or from the methadone clinic. Their mean (SD) age was 45.2 (7.9) years. Criteria for inclusion in the study were that patients were 18 years of age, met DSM-IV criteria for alcohol, cocaine, or opiate dependence, self-identified as African American and were abstinent. Exclusion criteria included mental retardation, dementia and acute psychosis. Patients were interviewed by a psychiatrist (AR) using the substance abuse section of the Structured Clinical Interview for DSM-IV (SCID) (Spitzer et al., 1995) to establish lifetime substance dependence diagnoses. The diagnosis of suicide attempt was implemented by A.R. after interviewing the patient with a standard series of clinical questions and reviewing all available collateral information, e.g. from mental health program staff, medical records, treating physicians. A suicide attempt was defined as a self-destructive act with some intent to end one's life that was not self-mutilatory in nature. Actions without suicide intent such as delicate self cutting and accidental drug overdose were excluded.

The second subsample was recruited as potential controls for genetic studies in substance dependence: 759 African American men ($N = 307$) and women ($N = 452$) recruited from a consecutive series of insulin-dependent diabetic outpatients seen at an ophthalmology clinic (54%) at the University of Medicine and Dentistry, New Jersey Medical School (UMDNJ), Newark, NJ and from churches and a blood bank in Newark, NJ, (46%). Their mean (SD) age was 34.3 (10.1) years. The diabetics underwent two physician-conducted structured interviews over a 6 year period that included questions about suicidal behavior and substance abuse (Roy, 2000). Any reports of suicidal behavior were discussed with the medical staff by AR. The participants recruited from churches and a blood bank underwent a semi-structured psychiatric interview conducted by a trained research worker (a former substance abuse counselor) that included a standard series of questions about lifetime suicidal behavior and substance abuse/dependence (reviewed by AR).

After a full description of the study was provided, all participants gave written informed consent to the study that was approved by the Institutional Review Boards of the VANJHCS and UMDNJ.

2.2. Childhood Trauma Questionnaire (CTQ)

The CTQ (28 item version) was completed by 538 patients (92% men) with substance dependence; 166 (90% men) had attempted suicide. The CTQ was completed by 342 non-substance dependent participants (42% men), 32 (28% men) of whom had attempted suicide (see Fig. 1).

The CTQ yields scores (5–25 each) for physical abuse, physical neglect, emotional abuse, emotional neglect, and sexual abuse, as well as a total score (25–125). Reliability and validity of the CTQ has been demonstrated, including in drug abusers and African American populations (Bernstein et al., 1994, 1997, 2003; Bernstein and Fink, 1998; Scher et al., 2001; Thombs et al., 2007). The CTQ was used as a continuous measure in all logistic regression analyses.

A dichotomous total CTQ score was derived solely to illustrate the direction of interaction effects on suicide attempt. A total CTQ score 1 SD above the mean CTQ score of non-substance dependent/no suicide attempt participants (36.49 (11.67), i.e. 48) was designated 'high trauma' ($N = 290$); lower total CTQ scores (<48) were designated 'low trauma' ($N = 590$). This approach has been used in our earlier studies: Enoch et al. (2010) and Roy et al. (2010).

2.3. Genotyping

Genomic DNA was isolated from blood using standard protocols. A genomic region containing sequence 5 kb upstream and 1 kb downstream of *CRHBP* was retrieved from NCBI Human Build 35.1. Haplotype-tagging SNPs, derived from the African HapMap sample, were identified using a previously described design pipeline (Hodgkinson et al., 2008). Eight *CRHBP* SNPs, listed in Table 1, were genotyped using the Illumina GoldenGate platform (Hodgkinson et al., 2008). Using the identical methodology, four *CRH*, nine *CRHR1* and eleven CRHR2 haplotype-tagging SNPs were genotyped (Table 1). All SNPs were in Hardy–Weinberg equilibrium other than *CRHBP* rs32897 ($p = 0.0001$) that was excluded from analyses.

2.4. Summary of dataset

An illustration of the dataset used in this study is provided in Fig. 1. DNA was available for a total of 830 participants: 141 suicide attempters and 689 non-suicide attempters. For G × E analyses, both DNA and CTQ scores were available for a total of 474 individuals, including 112 suicide attempters and 362 non-suicide attempters. Missing DNA and CTQ data was random and showed no selection bias.

2.5. Assessment of population stratification using ancestry informative markers

The samples were genotyped for 186 HapMap derived ancestry informative markers (AIMS) (Hodgkinson et al., 2008). The same AIMS were also genotyped in 1051 individuals from the 51 worldwide populations represented in the HGDP-CEPH Human Genome Diversity Cell Line Panel (http://www.cephb.fr/HGDP-CEPH-Panel). *Structure 2.2* (http://pritch.bsd.uchicago.edu/software.html) was run simultaneously using the AIMS genotypes from our sample and the 51 CEPH populations to identify population substructure and compute individual ethnic factor scores. This ancestry assessment identifies seven ethnic factors that have been previously described (Hodgkinson et al., 2008). In this sample of African Americans, the mean African factor score was 0.77 (median = 0.81) and the mean European factor score was 0.09 (median value = 0.04). Both a Mid East factor and an Asian factor had a mean score of 0.06 (median 0.04).

2.6. Statistical analyses

Logistic regression analyses with attempted suicide as the dependent variable were undertaken using JMP 7 software and yielded likelihood ratio (L-R) χ^2 results. Backward stepwise regression was performed with predictor variables being eliminated from the model in an iterative process if the level of significance was $p > 0.1$. The continuous CTQ score and European ethnic factor score and the dichotomous variables: age, sex and substance dependence diagnosis were initially included as predictor variables. Age and sex had no significant effects in any model. Substance dependence and the continuous CTQ score were included in all models since they had a significant effect.

There was a significant substance dependence × CTQ interaction for all SNPs, therefore this interaction term was included in all analyses. The genotype × CTQ interaction term was included where p 0.1. There was no substance dependence × genotype interaction ($p =$

0.5–0.9) nor was there any association between *CRHBP* SNP genotypes and substance dependence.

Backward stepwise regression was performed for each of the seven *CRHBP* SNPs, as shown in Table 2; likewise for the *CRH, CRHR1* and *CRHR2* SNPs.

In order to prevent spurious G × E results due to small sample sizes, for each of the SNPs that had minor allele frequencies (MAF) of 0.30 the minor homozygotes and heterozygotes were combined as one group.

Haplotype frequencies were estimated using a Bayesian approach implemented with PHASE (Stephens and Donnelly, 2003). Haploview version 2.04 Software (Whitehead Institute for Biomedical Research, USA) was used to produce linkage disequilibrium (LD) matrices. Since rare and uncommon haplotypes are subject to estimation errors because of increased sampling variance, all analyses were conducted with haplotypes 5% frequency. These haplotypes were included in models for *CRHR1* and for *CRH* together with the predictor variables listed above and backward stepwise regression was performed.

Based on earlier studies our *a priori* hypothesis was that we would find phenotypic associations within the distal region of *CRHBP*. Nevertheless, in the African Americans the five distal SNPs were not in LD and therefore we could not predict which of these SNPs might have an effect. Therefore we applied a False Discovery Rate (FDR) correction (Benjamini et al., 2001) for comparisons across the five SNPs. In contrast, based on earlier publications our hypothesis was that there would be no main or G × E effects on suicidality of the two proximal SNPs, rs3792738 and rs6453267 and therefore we applied FDR corrections for all 7 *CRHBP* SNPs. The analysis of additive effects of *FKBP5* and *CRHBP* variation was strongly hypothesis-driven and no corrections were warranted. For *CRHR1*, one haplotype block extended distally across the gene and included seven SNPs. There were two proximal SNPs that were not in LD and were therefore independent of each other and of the haplotype block. Therefore an adequate Bonferroni correction for these 2 SNPs would be $0.05/3 = 0.017$.

3. Results

Table S1 provides the mean (SD) total CTQ and subscale scores for the groups of participants who had and had not, attempted suicide. Fig. 2 shows that, irrespective of substance dependence and gender, suicidal behavior was associated with higher childhood trauma scores and that the proportion of women attempting suicide was numerically but not significantly greater compared with men in both the substance dependent group (0.40 vs 0.30, $p = 0.265$) and the non-substance dependent group (0.12 vs 0.06, $p = 0.524$).

3.1. CRHBP

There was no haplotype block structure in the African Americans (Fig. 3). Therefore analyses were conducted with the individual SNPs.

3.1.1. Main effects of CRHBP SNPs—Using the whole dataset of genotyped individuals ($N = 830$) and including the dichotomous 'substance dependence diagnosis' as a covariate, there was no main effect of any of the *CRHBP* SNPs on suicidal behavior.

3.1.2. Gene–environment (G × E) interactions—These analyses were conducted on the 474 participants who had both CTQ data and DNA (Fig. 1). From Table 2 it can be seen that within the whole logistic regression model there was a main effect of rs6453267. This variant allele, unique to individuals of African ancestry (Table 1), was more common in

non-suicide (0.23 vs 0.19, p = 0.007). The result remained of trend significance after FDR correction (p = 0.0504).

All the interactive analyses shown in Table 2 were performed with the total CTQ score, a continuous variable. There were nominally significant G × E interactive effects for rs7728378, rs10474485 and rs6453267 but after FDR correction, only the results for rs7728378 remained significant.

For rs7728378 and rs10474485 the major homozygote interacted with total CTQ score to predict suicide attempt. In order to illustrate the direction of this interactive effect on suicide attempt the dichotomous total CTQ score was used, the derivation of which was described earlier. Since the MAF was high (0.39) for rs10474485 we were able to identify the direction of effect of all three genotypes. The prevalence of suicide attempt in carriers of the major homozygote, heterozygote and minor homozygote was as follows: high trauma: 0.54, 0.27, 0.32; low trauma: 0.17, 0.20, 0.17.

3.1.3. G × E interactions: participants with substance dependence—The logistic regression analyses were repeated in the substance dependent individuals (N = 303). Even though the sample size was smaller than the total sample (N = 474) similar G × E results were obtained for the *CRHBP* SNPs. Substance dependent vs total sample: rs1500: p = 0.026 vs 0.078; rs10474485: p = 0.038 vs 0.032; rs7728378: p = 0.054 vs 0.0098. The main effect of rs6453267 remained significant: p = 0.037 (uncorrected).

3.2. Independent and additive effects of CRHBP and FKBP5

3.2.1. Independent effects—In order to test for an additive effect with *CRHBP* we selected the *FKBP5* SNP rs3800373 that had the greatest G × E interactive effect within the logistic regression model (L-R χ^2 = 6.6, 1df, p = 0.0103) in our earlier study (Roy et al., 2010). For this analysis we selected *CRHBP* rs7728378 that showed the strongest G × E result in the current study and in our earlier study (Enoch et al., 2008).

Fig. 4B illustrates the results of independent analyses in *FKBP5* rs3800373 and *CRHBP* rs7728378 using the derived dichotomous CTQ score. For the sake of simplicity we designated the *FKBP5* rs3800373 genotype groups as: major homozygote = FF; minor homozygote + heterozygote = ff. Likewise for the *CRHBP* rs7728378 genotype groups: major homozygote = CC; minor homozygote + heterozygote = cc. In the case of high childhood trauma, the prevalence of suicide attempt was 0.49 in carriers of the *FKBP5* rs3800373 major homozygote (FF) and 0.40 in carriers of the *CRHBP* rs7728378 major homozygote (CC) whereas the prevalence in carriers of the minor homozygote/heterozygote was 0.28 (ff) and 0.31 (cc) respectively. Without exposure to childhood trauma, genotype had no effect on suicide risk (prevalence = 0.17–0.20).

3.2.2. Additive effects—An analysis of the interaction of total CTQ score (continuous variable) with combined *FKBP5* rs3800373 and *CRHBP* rs7728378 genotypes for each individual was significant within the logistic regression model: L-R χ^2 = 11.6, 3 df, p = 0.0087. The interaction term contributed 2.4% of the variance within the total model. This represents the sum of the interaction term variance in the independent *FKBP5* rs3800373 (1.4%) and *CRHBP* rs7728378 (1.2%) models.

Fig. 4A illustrates the results using the derived dichotomous total CTQ score and shows that the prevalence of suicide attempt in individuals exposed to high childhood trauma was 0.35 in carriers of only the *FKBP5* rs3800373 major homozygote (cc/FF) and 0.30 in carriers of only the *CRHBP* rs7728378 major homozygote (CC/ff) however the prevalence increased to 0.58 in carriers of both major homozygotes (CC/FF). In contrast, individuals who were not

carriers of either major homozygote (cc/ff) were resilient to the effects of childhood trauma; the prevalence of suicide attempt was the same in these individuals (0.24) as in all other genotype groups who had not experienced childhood trauma (0.16–0.22).

3.3. Secondary analyses

3.3.1. CRHR1—As shown in Fig. 3, one haplotype block extended distally across the gene and included seven SNPs from rs110402 to rs17689918 (listed in Table 1). There were five haplotypes with 0.05 frequency accounting for 85% of the haplotype diversity (Fig. S1). There were no haplotype or diplotype associations with suicidality, no $G \times E$ interactive effects and no SNP associations.

Two SNPs, rs9900679 and rs4792887, were not in LD with any SNPs (Fig. 3, Fig. S1). Carriers of the rs9900679 variant allele that is unique to individuals of African ancestry (Table 1) were more likely not to have attempted suicide (0.07 vs 0.03; L-R χ^2 = 7.4, 3 df, p = 0.007). This result survived Bonferroni correction.

3.3.2. CRH—All four SNPs were in strong LD (Fig. 3). There were four haplotypes with 0.05 frequency that accounted for 99% of the haplotype diversity (Fig. S1). There were no haplotype or SNP associations or $G \times E$ interactive effects on suicidality.

3.3.3. CRHR2—There was no LD across the gene (Fig. 3). There were no main or $G \times E$ effects.

4. Discussion

In this study we investigated main effects of the *CRHBP* gene and $G \times E$ interactive effects on suicidal behavior in a sample of African American participants with substance dependence. We did indeed find nominally significant $G \times E$ effects for *CRHBP* SNPs rs7728378, rs10474485 and rs6453267 although after FDR correction only the results for rs7728378 remained significant. Other than rs6453267, which has a protective allele that is common in African Americans but is rare or absent in other ethnic groups, we did not detect any main effects of *CRHBP* variation on suicide risk.

The *CRHBP* SNPs rs7728378 and rs10474485 that were implicated in suicidal behavior showed significant or trend associations with anxiety disorders in Plains Indians and AUDs in U.S. Caucasians and were included in one distal haplotype block in these two samples (Enoch et al., 2008). The SNP rs7728378 that showed the strongest effect in our earlier study also showed the strongest interactive effect with childhood trauma in our current study. It is also noteworthy that the same rs7728378 allele was implicated in suicidal behavior in the African Americans and AUD in the U.S. Caucasians however the allele frequencies were very different: 0.77 in African Americans and 0.38 in U.S. Caucasians. The results from four other *CRHBP* studies (see Fig. 3) also lend support to our findings. In a study of schizophrenics, rs1875999 heterozygotes had a greater risk of attempting suicide (De Luca et al., 2010). Rs10474485 was associated with remission of depressive symptoms, predominantly in individuals with anxious depression (Binder et al., 2010) and with depressive symptoms in alcoholics in a study that genotyped the same SNPs as in the current study, using the same platform (Kertes et al., 2010). Finally, rs10055255, located within the distal region of *CRHBP* (Fig. 3), has been shown to moderate stress-induced craving in heavy drinkers (Ray, 2011). Thus the results of our and other studies provide mounting evidence that *CRHBP* variation may play a role in stress-related disorders.

Since 65–90% of total CRH is thought to be tightly bound to CRHBP (Behan et al., 1997), the effectiveness of CRH in stimulating ACTH to release cortisol might be influenced by

variation in *CRHBP* expression. The binding of cortisol to the GR and the translocation of the GR into the nucleus resulting in altered expression of stress-response genes is moderated by a large molecular complex. Of the genes encoding these proteins, only *FKBP5* has been shown to be associated with stress-related phenotypes (Binder et al., 2004). In an earlier study of this dataset we showed that the interaction between childhood trauma and *FKBP5* haplotypes and SNPs influenced suicidal behavior (Roy et al., 2010). The current study shows that an additive effect between *CRHBP* and *FKBP5* variation in the interaction with childhood trauma predicts risk for suicide attempt. Amongst individuals who had been exposed to high childhood trauma, carriers of either the *CRHBP* rs7728378 major homozygote or the *FKBP5* rs3800373 major homozygote had suicide attempt rates of 0.31–0.35 respectively but this rate was almost double (0.58) in individuals who carried both major homozygotes. In contrast, trauma-exposed individuals who carried neither major homozygote had the same suicide rate (0.24) as individuals not exposed to childhood trauma.

Some studies have shown interactive effects between childhood trauma (measured by the CTQ) and *CRHR1* variation, including SNPs genotyped in our study: rs110402 and rs242924, on adult depression/depressive effects (Bradley et al., 2008; Heim et al., 2009; Polanczyk et al., 2009), and cortisol response (Tyrka et al., 2009); and rs4792887 on suicidality in individuals with low stress exposure (Wasserman et al., 2008). The one finding of the current study was that the non-ancestral allele of *CRHR1* rs9900679, unique to African ancestry, was protective against suicide attempt. Since no studies have associated *CRH* or *CRHR2* variation with suicidality other than one study indicating a *CRHR2* microsatellite association with severity of suicidal behavior in bipolar disorder (De Luca et al., 2007), our negative findings for *CRH* and *CRHR2* were as predicted.

CRHBP is highly conserved across vertebrates and in humans encodes a 322 amino acid protein that contains 5 disulphide bonds that are essential for CRH binding (Westphal and Seasholtz, 2006). The *CRHBP* SNPs that have been associated with stress-related phenotypes in our and other studies are located at the 3′ end of the gene. At the present time, no evidence has been provided that any of the SNPs in this region are or are not functional although Binder et al. (2010) did show that the variant allele of a 3′ SNP rs10473984 was associated with higher ACTH concentrations and speculated that this might be due to reduced *CRHBP* expression. It is noteworthy that a second *CRHBP* isoform has been identified in brain in which the terminal exon is spliced out in favor of two alternative exons that lie 3′ to the gene resulting in a truncation of the protein at the C-terminus with the normal 52 amino acids being replaced by 18 novel amino acids (Enoch et al., 2008). This change in peptide sequence might affect protein folding and stability that might alter CRH binding affinity.

Studies in rats have established that poor maternal contact in early life, comparable to emotional neglect of children, results in HPA axis dysregulation and reduced expression of hippocampal GR gene *NR3C1* resulting from hypermethylation of the promoter region (Weaver et al., 2004). Likewise, a study of postmortem hippocampus showed that suicide victims exposed to childhood maltreatment had increased methylation of an *NR3C1* promoter and decreased *NR3C1* expression (McGowan et al., 2009). Similarly, it is conceivable (but speculative) that our *CRHBP* G × E findings might be explained by the effects of childhood trauma on epigenetic modifications that influence the expression of the alternative exons and 3′UTR that is the location of the SNP cluster identified in our study (Tilgner et al., 2009).

There are a few limitations to this study. The CTQ is a self-report questionnaire although it has been shown to have high reliability and validity (Bernstein et al., 1994, 1997, 2003;

Bernstein and Fink, 1998; Scher et al., 2001; Thombs et al., 2007). The nature and severity of each suicide attempt was not systematically recorded. Since the complete SCID was not administered, data about possible comorbid disorders such as depression, anxiety disorders and PTSD was not available. Although the sample size decreased to 474 (112 suicide attempters) when examining $G \times E$ interactions, it is important to note that the participants in this study had for the most part been exposed to considerable childhood trauma and this could increase the strength of $G \times E$ interactions. For example, using the dichotomous CTQ clinical cut-off scores that differentiate between the presence or absence of significant abuse/neglect (Walker et al., 1999) we found that only 27% of individuals did not meet criteria for significant abuse/neglect. Finally this study was performed in African Americans predominantly with substance dependence and it is not clear that the results can be generalized to other populations or other patient groups.

In conclusion, the results of the present study show that interaction between childhood trauma and *CRHBP* variation appears to increase the risk of suicidal behavior, at least in individuals with substance dependence. We also found main effects of a *CRHBP* and a *CRHR1* SNP on suicide attempts; both these SNPs are unique to African ancestry. Finally, there appears to be a biologically plausible additive effect between genetic variation in *CRHBP* and *FKBP5*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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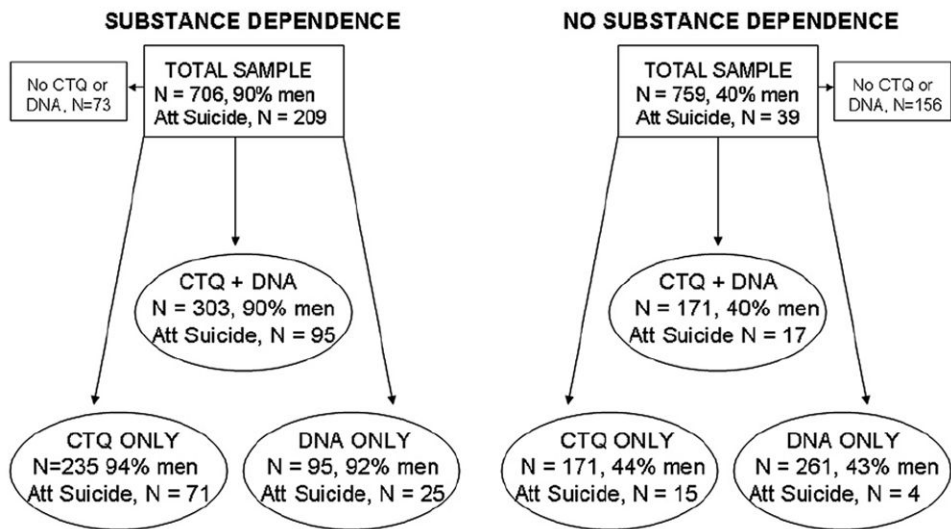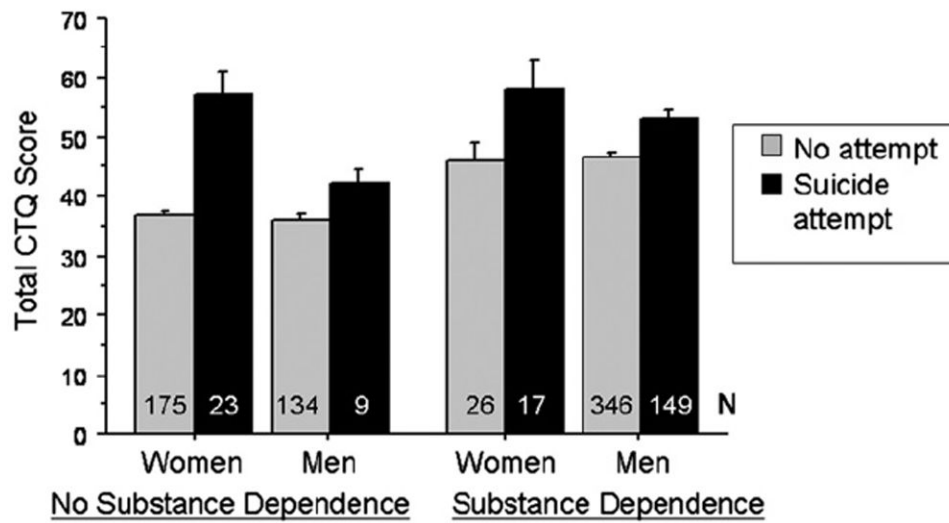
Fig. 1.
Flow chart detailing dataset.

Fig. 2.
Suicidal behavior is associated with increased childhood trauma in both substance dependent and non dependent men and women. Error bars are standard errors. N = number of individuals in each group.
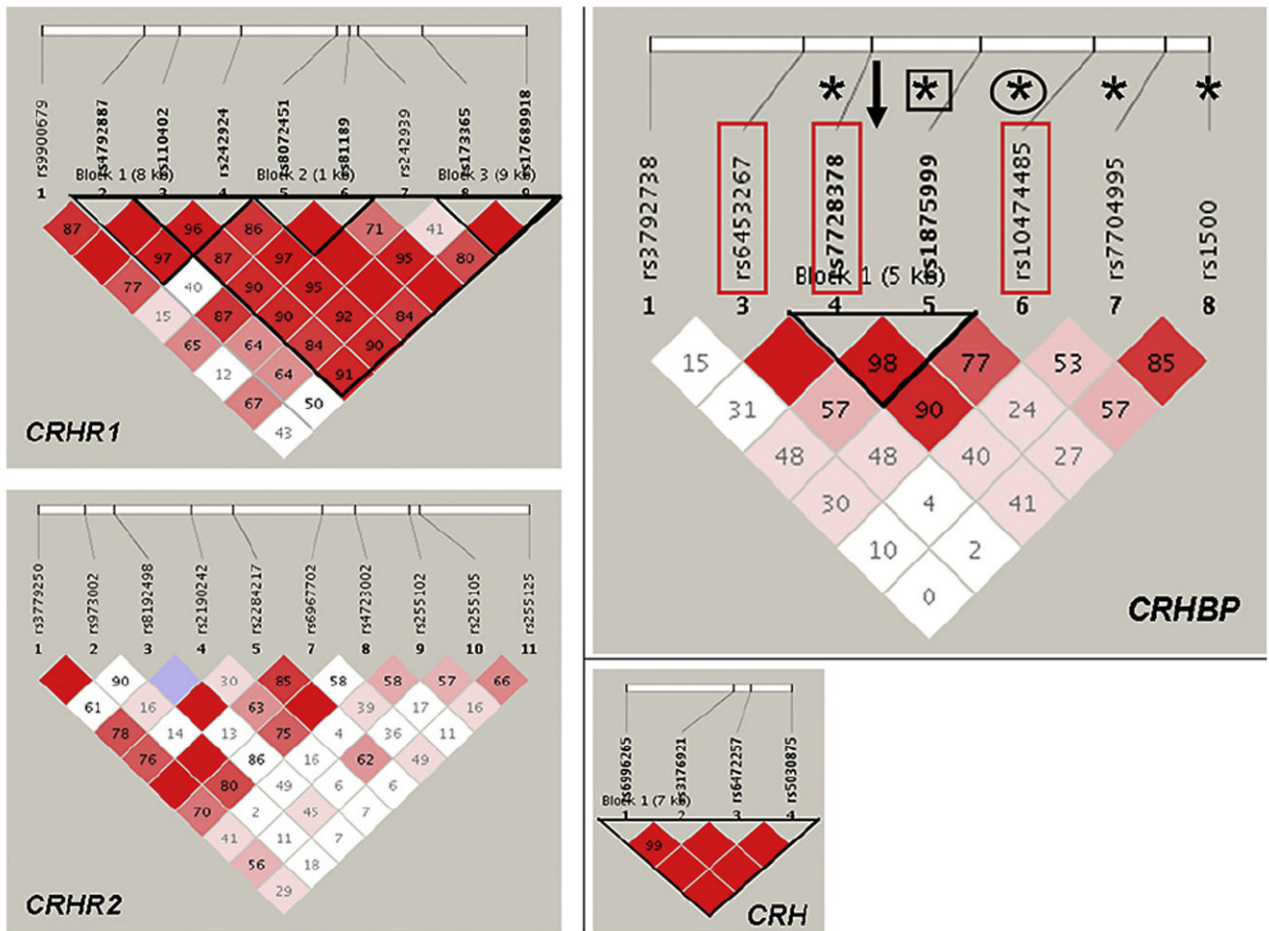
Fig. 3.
CRHBP, CRH, CRHR1 and *CRHR2* Haplotype block structure. The numbers in the squares refer to pairwise linkage disequilibrium measured as D'. Haplotype blocks were defined using a setting of average pairwise D' within-block of 0.80. *CRHBP*. The direction of gene transcription is from left to right. Rectangles denote SNPs that had a main or G × E effect on suicidal behavior in the current study. *denotes SNPs that had significant effects on alcohol use disorders, anxiety disorders and EEG alpha power (Enoch et al., 2008). Circle denotes a SNP implicated in depressive symptoms (Kertes et al., 2010, Binder et al., 2010) and square denotes a SNP implicated in suicide in schizophrenics (De Luca et al., 2010). An arrow indicates the location of a SNP associated with stress-induced craving in heavy drinkers (Ray, 2011).
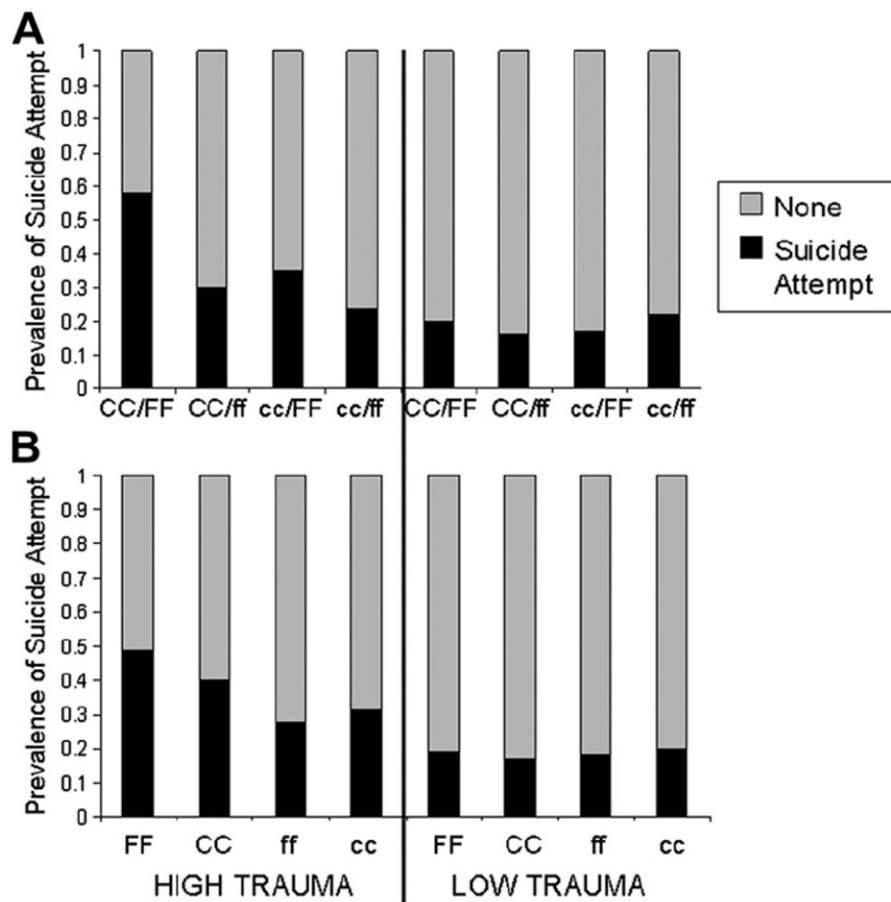
Fig. 4.
(A) Additive and (B) independent effects of *CRHBP* rs7728378 and *FKBP5* rs3800373 variation on suicide risk after exposure to high childhood trauma. *CRHBP* rs7728378: CC/cc genotypes; *FKBP5* rs3800373: FF/ff genotypes. CC, FF: major homozygote; cc, ff: minor homozygote + heterozygote. High level of childhood trauma defined as total CTQ score 1SD (48) above mean of non-substance dependent/no suicide attempt participants. Low level of childhood trauma defined as total CTQ score < 48. Panel A High Trauma: *N*'s: CC/FF (33), CC/ff (63), cc/FF (17), cc/ff (37); Panel A Low Trauma: *N*'s: CC/FF (60), CC/ff (109), cc/FF (47), cc/ff (68). Panel B High Trauma: *N*'s: FF (51), CC (103), ff (104), cc (61); Panel B Low Trauma: *N*'s: FF (108), CC (186), ff (181), cc (133). Note: the *N*'s do not correspond exactly since some individuals had genotypes for only one of the SNPs.

Table 1

The CRH gene complex: description of SNPs.

SNPs	Location		Base variation	MAF in AAs	Allele Freq[a] in HapMap, CEU–Asian
CRHBP	**Chr 5**	**Gene**			
rs3792738	76283540	5′ UTR	C > A	0.08	0.06–0.18
rs328967[b]	76286728	Intron	A > G	0.31	0.10–0.18
rs6453267	76291512	Intron	G > A	0.22	0.00–0.01
rs7728378	76295106	Intron	C > T	0.23	0.58–0.48
rs1875999	76300738	3′UTR	A > G	0.43	0.36–0.53
rs10474485	76306609	Alt isoform	C > A	0.39	0.18–0.37
rs7704995[c]	76310293	Alt isoform	C > T	0.30	0.65–0.43
rs1500	76312594	Alt isoform	C > G	0.30	0.65–0.44
CRH	**Chr 8**				
rs6996265	67248904	Intron	A > G	0.42	0.93–1.00
rs3176921	67253933	5′ region	C > T	0.41	0.87–1.00
rs6472257	67254734	5′ region	C > T	0.34	0.07–0.00
rs5030875	67256620	Intergenic	T > G	0.18	0.04–0.00
CRHR1	**Chr 17**				
rs9900679	41223923	Intron	G > C	0.06	0.00–0.00
rs4792887	41232791	Intron	C > T	0.33	0.07–0.00
rs110402	41235818	Intron	C > T	0.33	0.45–0.87
rs242924	41241147	Intron	C > A	0.32	0.45–0.87
rs8072451	41249496	Intron	C > T	0.07	0.19–0.00
rs81189	41250579	Intron	G > C	0.37	0.46–0.84
rs242939	41251360	Intron	A > G	0.31	0.07–0.00
rs173365	41256855	Intron	T > C	0.45	0.60–0.88
rs17689918	41265869	Intron	G > A	0.05	0.15–0.00
CRHR2	**Chr 7**				
rs3779250	30467500	Intron	G > A	0.15	0.70–0.49
rs973002	30472144	Intron	A > G	0.12	0.16–0.39
rs8192498	30475052	Val240Leu	G > A	0.02	0.00–0.00

SNPs	Location		Base variation	MAF in AAs	Allele Freq[a] in HapMap, CEU–Asian
CRHBP	Chr 5	Gene			
rs2190242	30482715	Intron	A > C	0.19	0.20–0.58
rs2284217	30486848	Intron	G > A	0.37	0.19–0.47
rs2014663	30488813	Intron	A > G	0.18	0.14–0.44
rs6967702	30495736	5′ region	G > C	0.10	0.00–0.00
rs4723002	30498941	Intergenic	A > G	0.16	0.08–0.22
rs255102	30504404	Intergenic	T > A	0.47	0.71–0.85
rs255105	30505347	Intergenic	T > C	0.18	0.71–0.61
rs255125	30516251	Intergenic	G > A	0.47	0.24–0.54

AAs = African Americans in this study (*N* = 830). The 3 distal *CRHBP* SNPs are located in an alternative isoform, expressed in brain.

[a]Frequency in HapMap European (CEU) and Asian populations of the minor allele in African Americans.

[b]This SNP was not in Hardy–Weinberg equilibrium and was therefore excluded from analyses.

[c]Now rs1715747.

Table 2

The influence of *CRHBP* SNPs and childhood trauma on attempted suicide.

SNPs	Gene effect		European ethnic factor		Childhood trauma effect		G × E interaction		Whole model	
	L-R χ^2	*p* value	L-R χ^2	*p* value	L-R χ^2	*p* value	L-R χ^2	*p* value	df	*p* value
rs3792738			2.8	0.094	29.3	**<0.0001**			5	**<0.0001**
rs6453267	7.2	**0.0072** [0.0504]	3.4	0.065	31.3	**<0.0001**	4.4	**0.0365** [0.0852]	6	**<0.0001**
rs7728378			4.4	0.035	23.8	**<0.0001**	6.7	**0.0098 [0.0490]**	6	**<0.0001**
rs1875999[a]			2.8	0.095	32.1	**<0.0001**	4.6	0.0986 [0.1232]	8	**<0.0001**
rs10474485[a]			2.7	0.100	29.8	**<0.0001**	6.9	**0.0320** [0.0800]	8	**<0.0001**
rs7704995			4.1	0.042	24.1	**<0.0001**	2.2	0.1396 [0.1396]	6	**<0.0001**
rs1500			4.0	0.047	26.6	**<0.0001**	3.1	0.0776 [0.1232]	6	**<0.0001**

Results are derived from logistic regression analyses for each *CRHBP* SNP and are for effect likelihood ratio (L-R) tests. Results for *p* 0.1 are presented. FDR adjusted *p* values are provided in parentheses. df = degrees of freedom. *N* = 474. Childhood trauma was measured as a continuous variable using the childhood trauma questionnaire (CTQ). Substance dependence (yes/no) was included as an independent variable in all SNP analyses (L-R $\chi^2 = 17.0$–22.1, $p < 0.0001$). The substance dependence × CTQ interaction was included in all analyses (L-R $\chi^2 = 5.7$–8.4, $p = 0.017$–0.004). The whole model analysis for each SNP accounted for 0.13–0.14 of the variance.

Bold values indicates significant findings.

[a] All 3 genotypes were included in analyses for these two SNPs since the MAF were >0.30. For the remaining SNPs the minor homozygotes and heterozygotes were combined to increase the power of the analyses.