

ORIGINAL RESEARCH

Genes involved in stress response and alcohol use among high-risk African American youth

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ABSTRACT

Background: Genetic and environmental factors influence substance use behaviors in youth. One of the known environmental risk factors is exposure to life stressors. The aim of this project is to study the interaction between *NR3C1* and *CRHBP*, genes thought to be involved in stress pathways, exposure to stressful life events, and adolescent alcohol use/misuse. **Methods:** The sample included 541 African American individuals (ages 13–18) from the Genes, Environment, and Neighborhood Initiative, a subset of the Mobile Youth Survey sample from whom DNA and more extensive phenotypic data were collected. Participants were selected from high-poverty neighborhoods in Mobile, Alabama, with potential exposure to a variety of extreme life stressors. **Results:** A measure of stressful life events was significantly predictive of alcohol use/misuse. In addition, this association was significantly dependent upon the number of putative risk variants at rs1715749, a single-nucleotide polymorphism (SNP) in *CRHBP* ($P \leq .006$). There was no significant interaction between *NR3C1* and stressful life events with respect to alcohol use/misuse, after taking into account multiple testing. **Conclusions:** These findings suggest that *CRHBP* variants are potentially relevant for adolescent alcohol use/misuse among African American youth populations being reared within the context of stressful life events and warrant replication.

KEYWORDS

Adolescents; alcohol; stressful life events

Introduction

Adolescent substance use is a major public health problem, with alcohol being the most widely used drug by youth.¹ Alcohol use among African Americans is an area of major concern because alcohol use is related to 3 of the 4 leading causes of death among African Americans between the ages of 12 and 20, including homicide, unintentional injuries, and suicide.² Further, despite lower rates of substance use among African American adolescents, they display more problematic trajectories of drinking as they age into adulthood and consequently report higher levels of substance-related problems than white Americans.³ For example, although African American youth are more likely to initiate smoking at a much later age than white youth, once they have initiated use, they are less likely to desist use.^{4,5} Consequently, African American youth are categorized as a population at greater risk for alcohol and substance use and misuse.⁶ For example, rates of heavy drinking and alcohol-related problems remain high in African American individuals aged 18 to 29 as compared with European Americans.⁷ African Americans are also more likely to face disadvantaged environmental conditions, such as poverty.⁶ Yet, there is a scarcity of research

examining adolescent substance use among African American youth living in high-poverty neighborhoods.⁸

Stressful life events

According to the National Comorbidity Replication Survey (NCS-R), 53% of adults have experienced some kind of major life stressor before the age of 18.⁸ Of these stressors, the most common consist of parental divorce, family violence, economic adversity, parental death, and mental illness. Although the biological stress response system is essential to human survival, it has been found that chronic or overactivation of the stress response system results in an increased vulnerability for not only physiological problems, but also an increased risk for psychopathologies such as anxiety, depression, and alcohol and other drug dependence.⁹

Adverse childhood events have been strongly related to alcohol use in early and mid-adolescence¹⁰ and to the subsequent development of alcohol dependence.¹¹ Stressful life events (SLEs) have also been linked to increased drug use over time among adolescents,^{12,13} and as a prominent predictor of early alcohol and drug use.¹⁴ Consequently, in recent years, the role

of SLEs has been an area of increasing interest because of its noted influence on substance use outcomes. However, the associations between SLEs and substance use in at-risk African American youth have received relatively little empirical attention. This represents an important gap in the literature because, in comparison with their white peers, African American youth experience higher rates of violence and poverty.¹⁵ In the few studies that have examined SLEs in African Americans, violent victimization was suggestive of playing an important role in prolonging substance use in a longitudinal study following African Americans aged 6 to 42.¹⁶ Furthermore, Doherty and colleagues¹⁶ also found that life traumas involving coercion and force can also be highly predictive of drug dependence among both Caucasian and African American populations.

Influence of genetic factors

Substance use not only is influenced by environmental factors, but is also a function of genetic factors.^{17–21} Further, studies have suggested that specific environmental factors can moderate the importance of genetic effects. Genes implicated in stress response are especially strong candidates for observing gene-environment interaction. For example, Covault and colleagues²² found that among college students being homozygous for the 5-HTTLPR short allele was associated with an increased risk for drinking outcomes (including drinking frequency and drinking intentions) if they had experienced multiple negative events in the past year relative to their counterparts who had low (or no) exposure to negative life events. The drinking of students homozygous for the long allele did not differ as a function of negative life events. These results parallel other findings of the interaction between the 5-HTTLPR short allele and childhood maltreatment exposure on use of alcohol in children.²³ Similarly, gene-environment interactions have also been observed with a variant of the gene for the dopamine type 2 receptor (*DRD2 Taq1* polymorphism). Madrid and colleagues²⁴ found that variability in stress exposure interacted with the *DRD2 Taq1* polymorphism in predicting risk for alcoholism, such that carriers of the A1 allele were at an increased risk for alcoholism when exposed to higher levels of stressors in comparison with lower levels of stressors. These results parallel findings by Bau and colleagues,²⁵ such that *DRD2 Taq1* A1 allele interacted with measures of stress to predict severity of alcoholism.

Another effort to extend the genotype-environment interaction literature included examining the role of *CRHR1*, which codes for the corticotropin-releasing hormone receptor in the pituitary gland. Interest in *CRHR1* as a candidate gene for the interaction between environmental stress and alcohol use resulted from animal studies.²⁶ The gene-environment interaction was also tested in a sample of 15-year-olds of predominantly European descent, selected from the Mannheim Study of Children at Risk. These results indicated that variation in the *CRHR1* gene and the greater number of negative life events during the previous 3 years was significantly associated with increasing rates of lifetime heavy alcohol use and levels of excessive use per occasion.²⁷ Another candidate gene includes *PER2*, which codes for the period circadian protein homolog 2 protein in humans. *PER2* is a circadian clock gene, which

influences the adaptation of an organism to its internal and external environment through governing circadian rhythms, which in turn has been found to be influenced by heavy alcohol use. Recent findings from the Mannheim study of Children at Risk indicated a protective effect of the minor allele of *PER2* on the susceptibility to alcohol use in young adults exposed to a higher number of stressful life events during the previous 3 years.²⁸

In this study, we examined the genes *NR3C1* and *CRHBP* based on their potential relevance for stress response. *NR3C1* codes for the glucocorticoid receptor that, when bound to glucocorticoids, acts as a transcription factor mediating the adaptation to environmental challenges and stress.²⁹ A number of functional polymorphisms have been identified that impact sensitivity or resistance to glucocorticoids,^{30,31} which are released following stress (including alcohol)-induced activation of the hypothalamic-pituitary-adrenocortical (HPA) axis. The known functional role of *NR3C1* variants in regulating the body's response to environmental challenges and psychosocial stress point to *NR3C1* as a high-priority candidate gene for this study. Laboratory studies have suggested a role for *NR3C1* in self-administration of drugs of abuse in animal models.²⁹

CRHBP codes for the corticotropin-releasing hormone-binding protein (CRH-BP), which regulates the availability of CRH to act at its receptors and inhibits CRH activation of the HPA axis. Activity of the CRH-HPA system has long been known to shape effects of environmental impacts during development (including SLEs and substance use) on responses to later life stressors and impact risk for psychiatric disorders.³² In a study by Ray,³³ it was found that a genetic variant in *CRHBP* was associated with variations in alcohol craving. Specifically, the T-allele homozygotes at rs10055255 (located within intron 6 of the *CRHBP* gene) reported greater alcohol craving during stress-induced conditions but not in the neutral conditions, greater negative moods following stress imagery but not after the neutral imagery, as well as greater stress-induced tension, compared with A-allele carriers. In addition, *CRHBP* variants have been associated with alcohol dependence occurring concurrently with 2 highly comorbid conditions that themselves are known to be linked with high stress exposure—anxiety³⁴ and depressive symptoms.³⁵

Objective of the present study

With the increasing documentation of the influence of specific environmental factors in moderating the importance of genetic effects, it is important to identify specific genes and environments that act together. The present study examined the associations among *NR3C1* and *CRHBP* genotypes and adolescent alcohol use/misuse in African American youth living in high-poverty neighborhoods as moderated by SLEs. Consistent with the theoretical mechanism outlined by Shanahan and Hofer, we expected genetic variance associated with alcohol use/misuse would increase under conditions of higher levels of SLEs.³⁶ Specifically, we predicted genetic effects would be most pronounced under conditions of high SLEs and attenuated under conditions of low SLEs.

Methods

Sample

The sample included individuals from the Genes, Environment, and Neighborhood Initiative (GENI). This group of individuals (ages 13–18) includes a subset of participants from the Mobile Youth Survey (MYS) sample from whom DNA was collected, as well as more extensive phenotypic data.^{37,38} The MYS is a community-based, multiple-cohort longitudinal study of adolescents who live in impoverished neighborhoods in Mobile, Alabama. The study began in 1998 with the goal of studying the etiology of risk behaviors among adolescents living in extreme poverty and how factors (such as family, school, and neighborhood) affect risk behaviors. The GENI study was developed with the primary aim of understanding gene-environment interplay for these risk behaviors.

Participation in GENI involved an extensive interview, using an audio computerized self-administered interview (ACASI) approach, for all eligible adolescents from GENI families and their caregiver. The interviews involved questions related to the primary outcomes of interest, including sexual risk taking, substance use, and externalizing problems as well as on exposure to stressors, neighborhood conditions, and other potential risk or protective factors. Additionally, select candidate genes were genotyped based on the existing literature, which connects these genes to the risk behaviors of interest to GENI. Consequently, GENI aimed to investigate the gene-environment interplay in relation to a variety of risk behaviors among African American youth in urban, high-poverty neighborhoods. The institutional review boards at Northwestern University, Virginia Commonwealth University, the University of Illinois at Chicago, and the University of Alabama approved procedures for this study.

The total GENI sample included 592 participants; however, there was a small group of individuals from whom responses were missing on key analytic variables. Therefore, this analytic sample represents 541 individuals (mean age [SD] = 15.89 [1.43]; 51.6% female).

Measures

Alcohol use

Data on alcohol use/misuse were collected from the AIDS Risk Behavior Assessment (ARBA) scale.^{39–44} A principal component analysis was conducted using 3 items based on frequency, quantity, and binge drinking: “In the last 12 months, how many days did you drink alcohol?” “Think of all the times you have had a drink during the last 12 months, how many drinks did you usually have each time?” and “Over the last 12 months, on how many days did you drink 5+ drinks in a row, within a couple of hours?” Standard definitions of “drinks” were provided. Factor scores were calculated on all participants in the sample, including those who did not endorse ever drinking. In this manner, initiation and use dimensions are collapsed together, which is more appropriate among a sample of this age group than is the case with a sample of adults.

Stressful life events

The Exposure to Stressors scale measures total amount of exposure to life stressors, as well as frequency of exposure to these stressors.^{45,46} This is a 16-item scale assessing items within 3 major categories: life transitions, circumscribed events, and exposure to violence during the last 12 months. Response options included “yes” (coded as 1) or “no” (coded as 0). Frequency of exposure to these stressors was assessed based on the event occurring “once,” “twice,” or “three or more times.” For the purposes of the present study, we used a sum score of SLEs. Previous studies have found that the joint effect of exposure to multiple adverse events is stronger than the effect of a single adverse event. For example, Pilowsky and colleagues¹¹ found that individuals who experienced 2 or more adverse childhood events are at increased risk for lifetime alcohol dependence.

Genotyping

DNA was obtained via saliva samples using Oragene collection kits under the supervision of a specially trained interviewer. Saliva samples were labeled anonymously and sent to the Virginia Institute for Psychiatric and Behavioral Genetics (Richmond, VA), where DNA extraction and genotyping occurred. In total, DNA samples have been obtained from 579 individuals, representing 98.3% of the total GENI sample.

A total of 18 single-nucleotide polymorphisms (SNPs) were genotyped across the 2 genes, all of which were based on HapMap data from the Nigerian Yoruba population, in order to capture the genetic variability in individuals of African descent. All SNPs were in Hardy-Weinberg equilibrium. Genotyping was conducted using fluorescence polarization detection of template-directed dye-terminator incorporation (FP-TDI) with appropriate AcycloPrime SNP detection kit for specific polymorphisms (PerkinElmer, Boston, MA) and an automated allele-scoring platform.⁴⁷

The genotyping success rate for *NR3C1* and *CRHBP* within this sample was >98% for all variants. Haploview⁴⁸ was used to estimate linkage disequilibrium (r^2) across the full set of genotyped SNPs (summarized in Figure 1). A multiple-testing correction across the SNPs was performed using the Web-based software SNPSpD,⁴⁹ which takes into account the number of SNPs genotyped and the linkage disequilibrium (LD) structure between them. Based on this test, we used adjusted significance values of $P = .006$ for *NR3C1* and $P = .007$ for *CRHBP* as evidence for association and interaction.

Statistical analyses

For these analyses, each of the SNPs was coded 0, 1, or 2, reflecting an additive genetic model. This coding is in reference to the number of copies of the minor allele (Table 1). We used bivariate correlations to assess the association between SLEs and alcohol use/misuse. We then used linear regression models in SPSS Statistics (version 20.0; IBM, Armonk, NY) to assess the additive and interactive effects for genotype and SLEs in predicting alcohol use/misuse. The interaction was modeled by creating cross-product terms between each SNP and total SLEs (centered on its mean to aid in interpretation). The covariates of child age and sex were accounted for in calculating the main

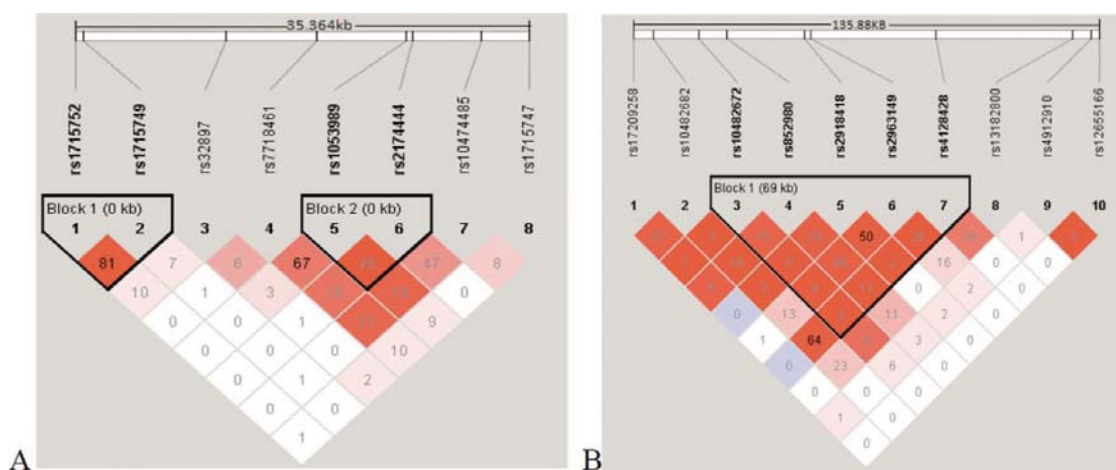


Figure 1. Haploview plot of linkage disequilibrium structure (r^2) across the genotyped single nucleotide polymorphisms in (A) *CRHBP* and (B) *NR3C1* using African Americans from the GENI sample.

effect. Covariates accounted for in calculating the interaction effects were sex and child age, the gene-specific SNP, and total SLEs. Regression models were conducted separately for each SNP.

In addition, simulations from recent research⁵⁰ demonstrate that using a cross-product interaction term with a 3-level genotype can lead to spurious results under some conditions and may not accurately capture the nature of the interaction. It has been suggested that a reparameterization of the regression equation with additional degrees of freedom is a better way to represent the nature of the interaction effects, rather than the single cross-product term that is more commonly used with a 3-category coding of the genotype. Therefore, we also fit an extended parameterization of the interaction model involving greater degrees of freedom, for SNPs that yielded interactions

with the single cross-product term, to determine whether the predicted interaction lines accurately represented the shape of the interaction in the data.

Results

Descriptive statistics

The number of participants who reported ever use of alcohol was 168 (31.1%). Of those who reported ever using alcohol, 59 participants (35.1%) reported drinking alcohol 1 to 2 days over the past 12 months, and 51 participants (30.3%) reported drinking alcohol 3 or more days in the past 12 months. An average of 2.40 drinks (SD = 1.39) was consumed per drinking occasion. For those subjects who reported drinking in the past

Table 1. Moderated multiple regression of *CRHBP* and *NR3C1* SNPs and total stressful life events predicting alcohol use/misuse in the whole sample.

SNP	SNP position	Relative position	Minor allele	Percentage of Subjects with copies of minor allele			Alcohol use/misuse			
				0	1	2	SNP main effect ^a		SNP × SLEs interaction ^b	
							B	P	B	P
<i>CRHBP</i>										
rs1715752	chr5:76274929	Promoter	T	36.3	46.5	17.2	0.05	.44	−0.04	.05
rs1715749	chr5:76275603	Promoter	T	31.5	47.4	21.1	0.02	0.73	−0.06	.006*
rs32897	chr5:76286728	Intron 3	C	49.6	42.0	8.4	0.17	.01	−0.01	.55
rs7718461	chr5:76293804	Intron 5	A	56.4	36.7	6.8	−0.14	.04	−0.01	.70
rs1053989	chr5:76300791	3' UTR	C	56.1	37.2	6.6	−0.14	.05	−0.01	.69
rs2174444	chr5:76301278	Downstream	T	33.2	50.2	16.6	0.02	.73	−0.02	.44
rs10474485	chr5:76306609	Downstream	A	35.4	49.7	14.9	0.03	.61	0.01	.60
rs1715747	chr5:76310293	Downstream	T	52.3	39.2	8.6	−0.08	.26	−0.02	.41
<i>NR3C1</i>										
rs17209258	chr5:142653590	Intron 7	G	91.0	8.8	0.2	−0.13	.40	0.02	.75
rs10482682	chr5:142659590	Intron 5	T	75.8	22.5	1.7	−0.03	.76	0.02	.47
rs10482672	chr5:142672726	Intron 3	A	64.6	31.5	3.9	−0.14	.08	−0.02	.36
rs852980	chr5:142681049	Intron 2	G	29.0	55.7	15.4	−0.05	.47	−0.01	.78
rs2918418	chr5:142703566	Intron 2	C	69.7	27.1	3.2	0.02	.84	−0.01	.73
rs2963149	chr5:142705277	Intron 2	T	50.7	42.0	7.3	0.03	.68	−0.01	.70
rs4128428	chr5:142742006	Intron 2	C	82.7	16.3	0.9	0.02	.88	0.02	.67
rs13182800	chr5:142781673	Intron 1	T	67.6	27.5	4.9	0.08	.30	0.03	.30
rs4912910	chr5:142787083	Intron 1	G	42.2	46.2	11.6	−0.09	.17	−0.01	.54
rs12655166	chr5:142789465	Intron 1	C	91.0	8.4	0.6	−0.03	.84	0.04	.49

^aCovariates accounted for in calculating the main effect were child age and sex.

^bCovariates accounted for in calculating the interaction effects were sex, child age, gene-specific SNP, and SLEs. The SLEs variable was centered on its mean for the analyses.

* $P \leq .007$ (Nyholt correction P value for *CRHBP*).

year, the number of participants who reported engaging in binge drinking (having 5 or more drinks in a row within a couple of hours) on 1 to 2 days over the last 12 months was 25 (22.7%), whereas 19 participants (17.1%) engaged in binge drinking on 3 or more days over the past 12 months. Overall, participants reported exposure to an average of 3.85 SLEs (SD = 3.08). There was a modest correlation between SLEs and alcohol use/misuse ($r = 0.19, P \leq .01$). An alcohol factor score was created such that higher scores indicated increased drinking frequency, quantity, and heaviness (range = -0.41 – 6.34 , mean [SD] = 0 [1]). The factor score explained 76% of the variance in alcohol use/misuse outcome. Factor loadings based on principal component analysis for the 3 items were 0.88, 0.90, and 0.83, respectively.

Regression analyses

Results of moderated multiple regressions of all SNPs and total SLEs predicting alcohol use/misuse are shown in Table 1. The main effect SLEs on predicting alcohol use and misuse was significant ($R^2 = 0.08, B = 0.06, P \leq .01$). There were no significant main effects of any of the SNPs on the outcome, based on the Nyholt correction.

In *CRHBP*, the interaction between SLEs and rs1715749 was significant in predicting alcohol use/misuse after applying the Nyholt correction ($R^2 = 0.09, B = -0.06, P \leq .006$) (Table 1). Post hoc analyses including a test of simple slopes for the significant interaction between SLEs and rs1715749 indicated that the association between SLEs and alcohol use/misuse was significant for the C/C genotypic group ($B = 0.11, P \leq .001$) and C/T genotypic group ($B = 0.06, P \leq .002$) but not for the T/T genotypic group ($B = 0.001, P = .97$). A regression plot to illustrate the interaction effect is shown in Figure 2A. After correcting for multiple testing, there were no significant interactions between SLEs and *NR3C1* (Table 1).

Figure 2B is a plot of the regression lines for each genotype from the reparameterization of the regression equation for rs1715749 in *CRHBP* that yielded a significant interaction effect

with the cross-product term. In a comparison of the regression plots representing the predicted values from the standard regression equation using the single cross-product term (Figure 2A) with the regression plots representing the extended parameterization (Figure 2B), we can see that using the single cross-product term relatively accurately captures the nature of the interaction in relation to the ordering of the genotypic categories and slope of the predicted regression lines for each genotype.

In a set of supplementary analyses, linear regression models were run among only those subjects who reported any drinking over the past year ($n = 168$). The main effect of SLEs on alcohol use and misuse remained significant ($R^2 = 0.08, B = 0.06, P \leq .05$) (Table 2). There were no significant main effects of any of the SNPs on the outcomes, based on the Nyholt correction. Although not significant after applying the Nyholt-adjusted P value, the interaction between SLEs and rs1715749 remained nominally significant in predicting alcohol use/misuse ($R^2 = 0.12, B = -0.09, P \leq .05$) (Table 2). Thus, even though the moderation effect is attenuated (as is expected given the reduced sample size), the effects observed in the full sample remain consistent when nondrinkers are excluded. This suggests that our results are not entirely driven by the distribution of the alcohol factor.

Discussion

The present study examined the associations between genotypes in *CRHBP* and *NR3C1* and adolescent alcohol use/misuse in a sample of African Americans adolescents living in high-poverty neighborhoods. We found a significant main effect of SLEs on alcohol use/misuse such that higher levels of SLEs were associated with higher levels of alcohol use/misuse. There was no evidence for main effects for either of the genes studied here after correcting for multiple testing. We found a significant interaction between rs1715749 in *CRHBP* and SLEs in predicting alcohol use/misuse. Individuals having 2 copies of the minor allele may be more resilient to environments of high SLEs than

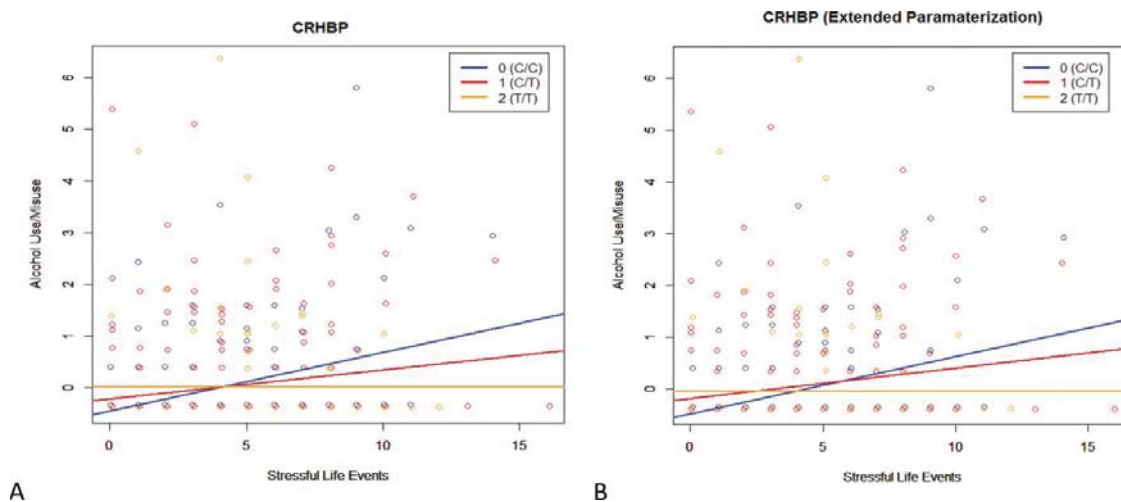


Figure 2. Regression plots. Evidence of the interaction effects for the significant SNP, rs1715749, in *CRHBP* of the (A) normal regression model and in the (B) extended parameterization model, including a comparison of the predicted values based on the regression equation to the raw data. The x-axis represents the raw values of SLEs to aid in interpretation. However, mean centered values of SLEs were used in the analyses.

Table 2. Moderated multiple regression of *CRHBP* and *NR3C1* SNPs and total stressful life events predicting alcohol use/misuse in individuals endorsing alcohol use in the past 12 months.

SNP	SNP position	Relative position	Minor allele	Percentage of subjects with copies of minor allele			Alcohol use/misuse			
				0	1	2	SNP main effect ^a		SNP × SLEs interaction ^b	
							<i>B</i>	<i>P</i>	<i>B</i>	<i>P</i>
<i>CRHBP</i>										
rs1715752	chr5:76274929	Promoter	T	31.6	50.6	17.7	0.02	.84	−0.08	.04
rs1715749	chr5:76275603	Promoter	T	27.1	52.9	20.0	−0.01	.95	−0.09	.02*
rs32897	chr5:76286728	Intron 3	C	46.2	41.1	12.7	0.18	.11	−0.08	.06
rs7718461	chr5:76293804	Intron 5	A	60.4	32.7	6.9	−0.26	.03	0.01	.76
rs1053989	chr5:76300791	3' UTR	C	59.1	33.3	7.5	−0.26	.03	0.01	.77
rs2174444	chr5:76301278	Downstream	T	34.6	50.3	15.1	0.11	.33	−0.06	.14
rs10474485	chr5:76306609	Downstream	A	35.2	48.4	16.4	0.06	.59	−0.03	.43
rs1715747	chr5:76310293	Downstream	T	58.2	34.8	7.0	−0.07	.56	0.02	.69
<i>NR3C1</i>										
rs17209258	chr5:142653590	Intron 7	G	92.4	7.6	0.0	−0.22	.47	0.1	.3
rs10482682	chr5:142659590	Intron 5	T	77.1	19.7	3.2	−0.06	.69	0.05	.39
rs10482672	chr5:142672726	Intron 3	A	66.3	29.4	4.4	−0.24	.08	−0.02	.59
rs852980	chr5:142681049	Intron 2	G	28.2	59.0	12.8	−0.07	.57	−0.04	.44
rs2918418	chr5:142703566	Intron 2	C	69.8	27.7	2.5	0.04	.8	−0.02	.61
rs2963149	chr5:142705277	Intron 2	T	49.4	43.8	6.9	0.05	.71	−0.05	.22
rs4128428	chr5:142742006	Intron 2	C	82.9	14.6	2.5	−0.02	.93	0.01	.86
rs13182800	chr5:142781673	Intron 1	T	63.5	31.4	5.0	0.07	.6	0.02	.64
rs4912910	chr5:142787083	Intron 1	G	44.7	48.4	6.9	−0.01	.93	−0.01	.92
rs12655166	chr5:142789465	Intron 1	C	90.0	9.4	0.6	−0.14	.56	0.07	.42

^aCovariates accounted for in calculating the main effect were child age and sex.

^bCovariates accounted for in calculating the interaction effects were sex, child age, gene-specific SNP, and SLEs. The SLEs variable was centered on its mean for the analyses.

**P* ≤ .05.

those individuals with 0 or 1 copy of minor allele in the same environment of high SLEs. Specifically, when we tested regions of significance for each genotype group contrast (i.e., 0 versus 1 copy of the minor allele), we found that individuals with 0 copies of the minor allele had significantly fewer alcohol use problems as a function of low SLEs than individuals with 2 copies of the minor allele in the same conditions of SLEs. These findings support the concept of differential susceptibility,⁵¹ which suggests that individuals who are highly susceptible to their environments fare more poorly in negative environments (i.e., high SLEs) but also fare much better in positive environments (i.e., low SLEs) compared with individuals who are less susceptible to their environments. After taking into account multiple testing, there were no significant interactions between any of the SNPs from *NR3C1* and SLEs in predicting alcohol use/misuse.

The SNP in *CRHBP* with which we find evidence of gene-environment interaction is novel in that this SNP has not been examined in relation to alcohol-related outcomes in prior research. The SNP is located in the promoter region of the gene and lies within a distinct haplotype block from SNPs in the 3' UTR (untranslated region) for which previous studies have evidenced associations with other psychiatric disorders.^{33,35} Among existing studies examining variants of *CRHBP*, Ray and colleagues³³ found that in a sample of non-treatment-seeking heavy drinkers, homozygotes for the T allele of rs10055255 reported higher stress-induced craving for alcohol. However, this SNP is not in LD with rs1715749.⁴⁸ Evidence for the role of *CRHBP* in stress and substance use also comes from animal models in which *CRHBP* in rat brain has been shown to modulate effects of corticotropin-releasing hormone on stress-induced relapse to drug abuse.⁵² Other studies have shown that *CRHBP* variants are potentially relevant for adolescent alcohol

use/misuse^{33,34}; however, we did not find any main effects in our African American population.

Limitations

The results from this study should be considered in the context of several limitations. First, the effects discussed in this study were identified within a sample of African American youth living in high-poverty neighborhoods; it is possible that the same findings might not be found within a different ethnic or socioeconomic sample. Drinking behaviors differ significantly across varying ethnic groups.⁷ Adolescents of European ancestry begin drinking at an earlier age and drink greater quantities with more frequency than adolescents of African ancestry.^{53–55} It is also noteworthy that endorsement of alcohol use is much lower among African American adolescents in comparison with their white counterparts, which has been suggested to be, in part, a result of greater levels of disapproval of substance use among African American populations.⁵⁶ Rates of alcohol use in this sample were comparable to the prevalence found nationally.⁵⁷ Specifically, ever drinking alcohol was endorsed by 30.7% of the participants in this sample, whereas the national prevalence for alcohol use in African American youth is 33.4%.⁵⁷ Rates of alcohol use in this sample were also comparable to other samples of African American youth living in public housing such that the prevalence of lifetime alcohol use among youth, ages 11 to 21, was reported as 35.3%.¹⁵ Accordingly, genetic effects associated with alcohol use may not be as easily detected in African American samples because of low endorsement. For example, in the present study, supplementary analyses were conducted among the subsample who endorsed any drinking over the past year. However, as expected, due to the reduced sample size owing to the low endorsement of alcohol use,

the interaction between SLEs and rs1715749 was nominally significant ($P < .05$) in predicting alcohol use/misuse, but did not remain significant after applying the Nyholt correction. Nonetheless, it is important to note that the effect size of the interaction between SLEs and rs1715749 in predicting alcohol use/misuse is larger than the resulting effect size seen when analyses included the whole sample, and that the direction of the effect of the interaction between SLEs and rs1715749 in predicting alcohol use/misuse remains the same as compared with the resulting interaction effect seen when analyses included the whole sample. Thus, future analyses run on samples of only those who endorse alcohol use in the past year would require larger sample sizes to address the limitation of low endorsement of alcohol use that is characteristic of African American adolescents.

Second, other issues affecting lack of consistency of effects seen across populations are differences in minor allele frequency.⁵⁸ Differing allele frequencies between populations can affect the ability to detect effects from one population to the next; therefore, studies of individuals of African descent may not detect the same signals (as seen in European Americans). Further, this may suggest that causal variants differ between populations of different ethnicities, thus highlighting the need to extend genotyping efforts to further elucidate potentially important effects that vary between populations. This underscores the importance of conducting genetically informative studies in African Americans and other minority populations.

Lastly, the present study examines only 2 genes involved in stress response. However, future investigations should include additional candidate genes that go beyond the commonly selected genes (e.g., 5-HTTLPR and DRD2). Other stress genes, such as *CRHR1* and *PER2*, have also been shown to interact with SLEs and alcohol-related outcomes, such as adolescent heavy alcohol use²⁷ and more drinks per occasion²⁸ in individuals of predominantly European descent. However, although not genotyped within this sample, the role of such additional stress-related genes should be included and extended to samples of African American youth in future analyses.

Conclusion

In conclusion, these results extend the growing literature on the role for *CRHBP* in substance use and other stress-related disorders. Notably, these findings suggest that *CRHBP* rs1715749 may contribute toward the risk of alcohol use/misuse in African Americans, such that the effect of this gene on alcohol use/misuse can vary as a function of exposure to SLEs. Identifying how specific environmental variables interact with genetic variants to influence substance use behaviors is necessary to develop a better understanding of the etiology of complex behaviors. Identifying environmental risk factors to developing problematic substance use outcomes also promotes a better understanding of those social contexts under which genetic predispositions are expressed. In addition, such interactions also aid in developing more targeted prevention and treatment programs for adolescent individuals at risk for developing substance use problems and/or for adolescents exposed to a wide variety of stressors. Initial analyses of genotype-intervention interaction studies suggest that children who are most at risk for substance

use and externalizing outcomes may also be those who are most likely to benefit from intervention.^{59–61}

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Author contributions

B. Mustanski was responsible for the study concept and design. F. Aliev and J. E. Salvatore aided in performing the statistical analysis. J. E. Salvatore and D. M. Dick aided in drafting the manuscript. J. E. Salvatore and D. M. Dick shared senior authorship responsibilities for these analyses and the manuscript. All coauthors aided with the interpretation of the results and provided critical revision of the manuscript for important intellectual content. All authors reviewed the content and approved the final version for publication.

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