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H2 haplotype at chromosome 17q21.31 protects against childhood sexual abuse-associated risk for alcohol consumption and dependence

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ABSTRACT

Animal research supports a central role for corticotropin-releasing factor (CRF) in actions of ethanol on brain function. An examination of alcohol consumption in adolescents reported a significant genotype × environment (G × E) interaction involving rs1876831, a corticotropin-releasing hormone receptor 1 (*CRHR1*) polymorphism, and negative events. *CRHR1* and at least four other genes are located at 17q21.31 in an extremely large block of high linkage disequilibrium resulting from a local chromosomal inversion; the minor allele of rs1876831 is contained within the H2 haplotype. Here, we examine whether G × E interactions involving this haplotype and childhood sexual abuse (CSA) are associated with risk for alcohol consumption and dependence in Australian participants ($n = 1128$ respondents from 476 families) of the Nicotine Addiction Genetics project. Telephone interviews provided data on DSM-IV alcohol dependence diagnosis and CSA and enabled calculation of lifetime alcohol consumption factor score (ACFS) from four indices of alcohol consumption. Individuals reporting a history of CSA had significantly higher ACFS and increased risk for alcohol dependence. A significant G × E interaction was found for ACFS involving the H2 haplotype and CSA ($P < 0.017$). A similar G × E interaction was associated with protective effects against alcohol dependence risk (odds ratio 0.42; 95% confidence interval 0.20–0.89). For each outcome, no significant CSA-associated risk was observed in H2 haplotype carriers. These findings support conducting further investigation of the H2 haplotype to determine the gene(s) responsible. Our results also suggest that severe early trauma may prove to be an important clinical covariate in the treatment of alcohol dependence.

Keywords alcohol dependence, association, childhood sexual abuse, *CRHR1*, haplotype, interaction.

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INTRODUCTION

Alcohol use disorders are common illnesses that profoundly impact the lives of affected individuals, their families and those with whom they interact (World Health Organization 2004; Hasin *et al.* 2007). The heritability of alcohol dependence has been estimated to be at least 50% by large twin studies (Heath *et al.* 1997; Prescott & Kendler 1999; Tsuang *et al.* 2001; Knopik *et al.* 2004), with the remaining contribution to liability attributed to individual-specific environmental sources. Included among genes implicated in genetic studies are those whose products are involved in the metabolism [*ADH* clusters (Luo *et al.* 2005b, 2006; Edenberg *et al.* 2006; Macgregor *et al.* 2009)] and effects [*GABRA2* (Covault *et al.* 2004; Edenberg *et al.* 2004; Lappalainen *et al.* 2005; Fehr *et al.* 2006), *CHRM2* (Wang *et al.* 2004; Luo *et al.* 2005a)] of ethanol; however, replicated findings to date explain only a minority of the overall genetic risk.

Caspi *et al.* (2002, 2003) influential examinations of other psychiatric disorders have stimulated more recent investigations of genotype × environment (G × E) interactions targeting alcohol-related phenotypes (Covault

et al. 2007; Kaufman *et al.* 2007; Armeli *et al.* 2008; Blomeyer *et al.* 2008; Ducci *et al.* 2008; van der Zwaluw *et al.* 2009). These studies have focused on environmental covariates occurring during important periods of brain development [physical abuse, childhood sexual abuse (CSA) (Kaufman *et al.* 2007; Ducci *et al.* 2008)] or acquisition of risky drinking patterns [parental rule-setting (van der Zwaluw *et al.* 2009), negative life events during adolescence (Blomeyer *et al.* 2008) or college (Covault *et al.* 2007; Armeli *et al.* 2008)].

One longitudinal investigation (Blomeyer *et al.* 2008) recruited as infants a high-risk sample enriched for those whose births involved severe obstetric problems or whose families experienced substantial psychosocial adversity. At age 15, these individuals ($n = 280$) completed an assessment that included measures of alcohol consumption and severe stressors experienced during the past 3 years. The authors found evidence of a significant G × E interaction involving a corticotropin-releasing hormone receptor 1 (*CRHR1*) polymorphism, rs1876831, and negative life events. Specifically, one or more copies of the minor allele was associated with reduced risk for (any) lifetime history of binge drinking and maximum amount of alcohol consumed per occasion in those who had experienced severe stressors. No evidence of a similar G × E interaction was seen for a second *CRHR1* polymorphism, rs242938. A prior examination (Treutlein *et al.* 2006) of their sample reported significant main effects on alcohol consumption for both of these *CRHR1* polymorphisms with confirmation in an older alcohol-dependent clinical sample. The potential importance of these findings is underscored by an extensive animal literature (Le *et al.* 2000; Hansson *et al.* 2006; Heilig & Koob 2007; Pastor *et al.* 2008; Sommer *et al.* 2008) supporting CRF_1 receptor involvement in diverse effects of ethanol including sensitization (Pastor *et al.* 2008), consumption (Hansson *et al.* 2006), withdrawal (Sommer *et al.* 2008) and stress-induced relapse (Le *et al.* 2000).

CRHR1 is located in a region, 17q21.31, which was recently described (Pennisi 2008) as 'one of the most structurally complex and evolutionarily dynamic regions of the genome'. A nearby gene, microtubule-associated protein tau (*MAPT*), first (Hutton *et al.* 1998) drew research attention to this area as a result of accumulating evidence (Pastor *et al.* 2004; Pittman, Fung & de Silva 2006) for association with risk of progressive supranuclear palsy (PSP), a neurodegenerative disease in which tau-positive neurofibrillary tangles are present. Additional examination (Pastor *et al.* 2004; Stefansson *et al.* 2005) revealed the existence of an extremely large linkage disequilibrium (LD) block spanning ~1.5 Mb extending across five adjacent genes (including *MAPT* and *CRHR1*). Two haplotypes, termed H1 and H2, have been described; the H2 haplotype contains a ~970 kb inversion that prevents recombination in H1/H2 heterozygotes (Stefansson *et al.* 2005). The H1 haplotype, present in all populations [H2 is found predominately in those of European ancestry (Stefansson *et al.* 2005)], is associated with increased risk for PSP and other neurological disorders (Pastor *et al.* 2004; Skipper *et al.* 2004; Cruts *et al.* 2005; Pittman *et al.* 2006; Sundar *et al.* 2007; Webb *et al.* 2008). Evidence of positive selection for the H2 haplotype was observed in a large Icelandic sample (Stefansson *et al.* 2005); however, a predisposition to a microdeletion syndrome resulting in mental retardation and neurological symptoms has also been associated with the H2 haplotype (Koolen *et al.* 2006).

A recent study (Tantisira *et al.* 2008) found an association with inhaled corticosteroid response in asthma for four single nucleotide polymorphisms (SNPs) that tag the H2 haplotype in samples from populations in which a similar association had been previously attributed (Tantisira *et al.* 2004) to rs1876828, a *CRHR1* SNP. Similarly, the minor allele of rs1876831, the *CRHR1* polymorphism for which a significant G × E interaction associated with risk for alcohol consumption was previously reported (Blomeyer *et al.* 2008), is also contained within the H2 haplotype. Research on this region has also not yet been integrated into the psychiatric literature.

The current report examines whether a similar G × E interaction is observed involving the H2 haplotype and history of CSA that is protective against alcohol consumption and DSM-IV alcohol dependence in the Australian sample of the Nicotine Addiction Genetics (NAG) project (Saccone *et al.* 2007; Agrawal *et al.* 2008). Our large sample, drawn from a heavy-drinking population, enriched for regular smokers [rates of regular smoking in clinical samples of alcoholics approach 90% (Hurt *et al.* 1996)] and having largely survived the period of greatest risk for the onset of alcohol dependence, is particularly well suited for this investigation.

MATERIALS AND METHODS

Sample ascertainment and recruitment

The Australian component of the NAG project (Saccone *et al.* 2007), a collaboration between Queensland Institute of Medical Research and Washington University School of Medicine investigators, used data collection procedures approved by both institutions' institutional review boards. Detailed descriptions of study methods have been reported (Saccone *et al.* 2007; Agrawal *et al.* 2008). In brief, prior reports by index cases of smoking status and family structure (in surveys of two large Australian Twin Panel cohorts and of spouses of the older twin cohort) were used to ascertain families with a sib pair [containing at most one monozygotic (MZ) twin] concordant for heavy smoking. Index cases were con-

tacted to confirm smoking history and to obtain permission to contact family members. Families with two available parents were prioritized. When both parents were not available, at least one unaffected sibling was targeted for recruitment. After confirmation of family eligibility, study materials (including consent form) were mailed and telephone interviews scheduled with individual family members. Interviewing was conducted from 2001 to 2006. Data from only one member of any MZ twin pair, the designated index case, were included in analyses.

Assessment

All participants first provided verbal consent. A computer-assisted telephone interview adapted from the Semi-Structured Assessment for the Genetics of Alcoholism (Bucholz *et al.* 1994) was then administered. Data collected included demographic information, DSM-IV diagnoses of psychiatric and substance dependence disorders, and other non-diagnostic sections. To reduce respondent time commitment, younger cohort twins (born 1964–1975) and spouses of older cohort twins were not re-administered some interview items identical to their prior assessment.

Two primary outcome measures are used in analyses reported here: (1) a quantitative alcohol consumption factor score (ACFS) (Agrawal *et al.* 2009; Grant *et al.* 2009); and (2) a binary DSM-IV diagnosis of alcohol dependence. Individuals who denied any lifetime alcohol use were coded as missing for the alcohol consumption measure and for alcohol dependence. Respondents who endorsed at least three of seven diagnostic criteria in a single year were given a DSM-IV alcohol dependence diagnosis. The ACFS was created from four indices of alcohol consumption queried in the alcohol use disorders section: (1) lifetime maximum 24-hour alcohol consumption (log-transformed to adjust for skewness); and for the heaviest drinking period of at least 1 year's duration, (2) weekly alcohol consumption (log-transformed); (3) frequency of drinking to intoxication; and (4) frequency of drinking = 5 drinks per day. Because the NAG sample is enriched for heavy smokers, data from BigSib, a general community sample ascertained from the Australian Twin Registry on the basis of large sibship size [see Saccone *et al.* (2007) for more information] were used to generate scoring coefficients more representative of the general population. An option in the Factor Procedure of SAS (SAS Institute 2004) enabled separate scoring coefficients to be calculated for women and men using BigSib sample data. The SAS Score Procedure was then used to apply these scoring coefficients to data from NAG project participants to obtain factor scores. Consistently high factor loadings for all component items were found for women (0.68–0.85) and men (0.63–0.92).

The primary covariate, CSA, is derived from a question in the conduct disorder section of the interview (not administered to parents, for whom data were thus not included in the current analyses): 'Before age 18, were you ever forced into sexual intercourse or any other form of sexual activity?' A follow-up question determined the age at which forced sexual activity first occurred. Two respondents ($n = 2$) who endorsed the forced sex question, but reported its first occurrence at an age = 18, were excluded from analyses (Nelson *et al.* 2002, 2006). Seven individuals whose responses to this item at subsequent assessment were inconsistent (three endorsed and then denied; four denied and then endorsed) were also excluded from further analyses. As we have done previously (Nelson *et al.* 2002, 2006), those reporting forced sexual activity with first occurrence before age 18 ($n = 151$) or who did not report ($n = 5$) age of first occurrence were coded as having a history of CSA.

SNP genotyping and haplotype assignment

DNA was extracted from blood samples by salting out. MassARRAY iPLEX technology (Sequenom, San Diego, CA, USA) was used for SNP genotyping. Polymerase chain reaction (PCR) primers, extension primers and multiplexing capabilities were determined with the Sequenom MassARRAY Assay Designer software v3.1.2.2 (Sequenom, San Diego, CA, USA). Standard procedures were used to amplify PCR products; unincorporated nucleotides were deactivated with shrimp alkaline phosphatase. A single base pair extension step was completed with the mass extension primer and the terminator (iPLEX). The primer extension products were cleaned with resin and spotted onto a silicon SpectroChip (Sequenom, San Diego, CA, USA). The chip was scanned with a mass spectrometry workstation (Bruker AXS, Karlsruhe, Germany). The resulting genotype spectra were analyzed with the SpectroTYPER software v3.4 (Sequenom, San Diego, CA, USA).

The 13 genotyped *CRHR1* SNPs included those from prior reports (Treutlein *et al.* 2006; Blomeyer *et al.* 2008; Bradley *et al.* 2008) and nearby SNPs identified from dbSNP. Hardy-Weinberg equilibrium (HWE) *P*-values were > 0.1 for all SNPs; no evidence of substantial Mendelian errors was found. The call rate for rs3029044, an insertion/deletion polymorphism, was 94%; call rates for the SNPs otherwise ranged from 0.95 to 0.98 (Table 1). We examined the LD relationships of the 13 SNPs (shown in Fig. 1 as pairwise r^2 values) and recognized that the large block of eight SNPs (including rs1876831) in very strong LD with minor allele frequencies ranging from 0.21 to 0.22 (Table 1) are part of the H2 haplotype. The other five SNPs forming two small additional LD blocks

Table 1 Genotyped *CRHR1* SNPs, minor alleles, MAFs and call rates.

CRHR1 *SNP*	*Minor allele*	*MAF*	*Call rate*
rs1876830	**T**	**0.22**	**0.98**
rs16940674	**T**	**0.22**	**0.98**
rs1876831	**T**	**0.22**	**0.97**
rs3029044	**Insert**	**0.22**	**0.94**
rs1396862	**A**	**0.22**	**0.98**
rs1912151	**T**	**0.21**	**0.97**
rs242938	A	0.06	0.98
rs2316764	**G**	**0.22**	**0.98**
rs242939	C	0.06	0.98
rs2316763	**T**	**0.22**	**0.98**
rs242924	T	0.42	0.98
rs110402	A	0.41	0.95
rs7209436	T	0.40	0.95

White (in bold) = H2 haplotype SNPs; grey = SNPs in two LD blocks (light and dark) outside of H2 not included in analyses.
CRHR1 = corticotropin-releasing hormone receptor 1; LD = linkage disequilibrium; MAF = minor allele frequency.

apart from the H2 haplotype are not included in the current analyses. Data were coded consistent with the prior report (Blomeyer *et al.* 2008) to enable comparison of individuals with one or more copy of the H2 haplotype to individuals homozygous for the H1 haplotype (i.e. consistent with a dominant mode of inheritance).

Genotyping results were entirely consistent for 1119 participants: for 671, no minor alleles were detected at any successfully genotyped locus (H2 haplotype absent); for 448, a minor allele was detected at each locus (H2 haplotype present). For another nine individuals, genotyping results included minor inconsistencies that primarily involved the more difficult to genotype rs3029044 insertion/deletion: six who were homozygous for the major allele at all but one successfully genotyped locus were coded as H2 haplotype absent; three others who were homozygous for the major allele at a single locus (with minor alleles present at the other successfully genotyped loci) were coded as H2 haplotype present. Finally, three individuals who had intermediate genotypic results were coded as indeterminate for H2 haplotype and excluded from subsequent analyses. Haplotypes were similarly assigned for all additional family members (including parents) with genotypic data available, and PedCheck (O'Connell & Weeks 1998) was used to look for Mendelian errors (total $n = 1814$). No Mendelian errors in haplotype assignment were identified. The final sample ($n = 1128$ individuals from 476 families) consisting of all those with data available for H2 haplotype, CSA and outcome measures included 565 women [mean age 41.0 (SD 8.6)] and 563 men [mean age 43.1 (SD 9.4)]. These individuals almost exclusively report Anglo-Celtic or other European ancestry.

Statistical analyses

Analyses were performed using the SAS statistical software package v9.1 (SAS Institute 2004). The primary analyses examine whether G × E interactions involving the H2 haplotype and a history of CSA are protective against alcohol consumption and DSM-IV alcohol dependence. For linear regression analyses, the SurveyReg Procedure was used to control for inclusion of data from multiple members of families. For logistic regression analyses, the SurveyLogistic Procedure provided similar control. We performed *t*-tests to determine if mean ACFS differed by CSA status when controlling for gender and genotype. A significance threshold of alpha = 0.05 was used for all analyses.

RESULTS

Descriptive analyses

One hundred twenty-one women (21.4%) and 35 men (6.2%) reported a history of CSA; mean age at first CSA occurrence was 11.0 years (SD 4.3). A lifetime DSM-IV diagnosis of alcohol dependence was more common in men (40.7%) than women (21.2%). The mean ACFS values for women and men were 0.42 (SD 1.01) and 0.43 (SD 0.91), respectively. ACFS were correlated ($P < 0.0001$) with lifetime alcohol dependence diagnoses in both women ($r = 0.54$) and men ($r = 0.45$). Those with a history of CSA had significantly higher ACFS [mean values: CSA+ 0.67 (SD 1.10); CSA– 0.39 (SD 0.94); $P < 0.0031$].

Linear regression analyses examining ACFS

In linear regression analyses with ACFS as the dependent variable, we first confirmed that a history of CSA is associated with higher lifetime alcohol consumption (main effect for CSA, $P < 0.003$). We performed a similar analysis and found no significant main effect for H2 haplotype ($P > 0.77$). We then examined whether a G × E interaction involving CSA and H2 haplotype was observed in analyses that also included terms for main effects of CSA, gender and H2 haplotype. We found a significant G × E interaction with the H2 haplotype protecting against CSA-associated effects on alcohol consumption (see Table 2). To demonstrate the protective effects of the G × E interaction more clearly, we compared mean ACFS by CSA and H2 haplotype status. We initially confirmed that mean ACFS did not vary by gender for either H1 homozygotes ($P > 0.69$) or individuals with the H2 haplotype ($P > 0.88$). We then found that significantly higher ($P = 0.0006$) mean ACFS was associated with a history of CSA only in H1 homozygotes; in individuals with the H2 haplotype, the mean ACFS varied minimally ($P = 0.77$) with CSA status (Fig. 2).

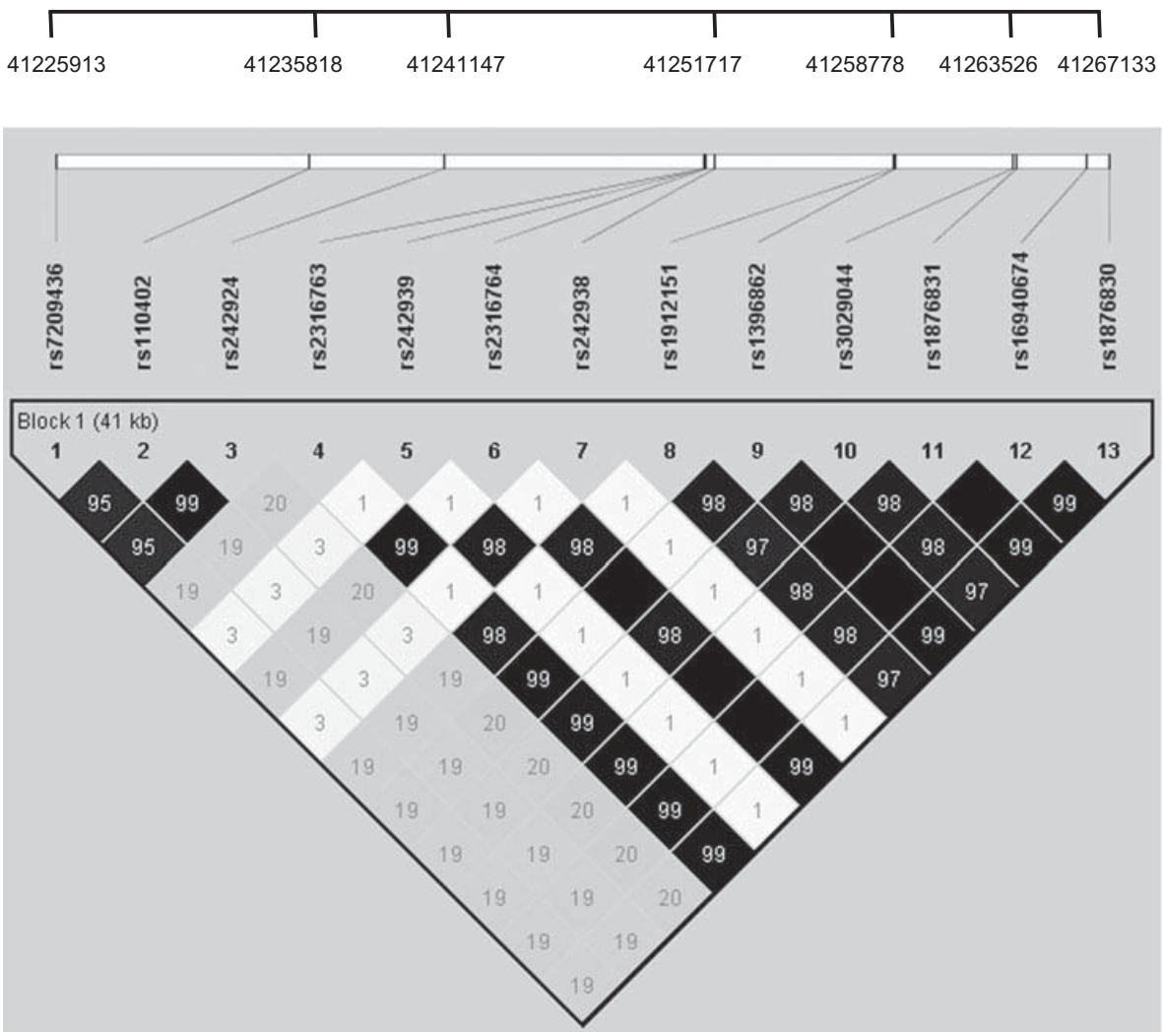

Figure 1 Linkage disequilibrium pattern (r^2 values shown) and physical location of genotyped *CRHR1* SNPs

Table 2 Contribution to ACFS of H2 haplotype, CSA and interaction controlling for gender ($n = 1128$).

Parameter	*Beta (95% confidence interval)*
Intercept	**0.33 (0.23 to 0.43)**
Male	0.06 (–0.06 to 0.17)
H2 haplotype	0.07 (–0.05 to 0.20)
CSA	**0.47 (0.22 to 0.71)**
CSA × H2 haplotype	**–0.41* (–0.75 to –0.08)**

Significant results are in **bold**; *$P < 0.017$.
ACFS = alcohol consumption factor score; CSA = childhood sexual abuse.

Logistic regression analyses examining alcohol dependence

We conducted a series of logistic regression analyses, controlling for gender, with alcohol dependence diagnosis as the dependent variable. We first confirmed that a history of CSA is associated with lifetime alcohol dependence risk [odds ratio (OR) 2.03; 95% confidence interval (CI) 1.40–2.92]; we again found no evidence of a significant main effect for H2 haplotype on risk for alcohol dependence (OR 0.92; 95% CI 0.70–1.21). We next included a term for the G × E interaction involving CSA and the H2 haplotype in a model that also contained terms for main effects of CSA history, gender and H2 haplotype. We found evidence of a significant G × E interaction for the H2 haplotype protecting against CSA-associated effects on alcohol dependence risk (OR 0.42; 95% CI 0.20–0.89; $P = 0.023$). We calculated alcohol dependence risk by CSA status, controlling for gender, separately for individuals with and without the H2 haplotype. For H1 homozygotes ($n = 677$), significant alcohol dependence risk was associated with CSA (OR 3.37; 95% CI 2.03–5.59). For those with the H2 haplotype ($n = 451$), no evidence was found of CSA-associated risk (OR 1.04; 95% CI 0.57–1.90).

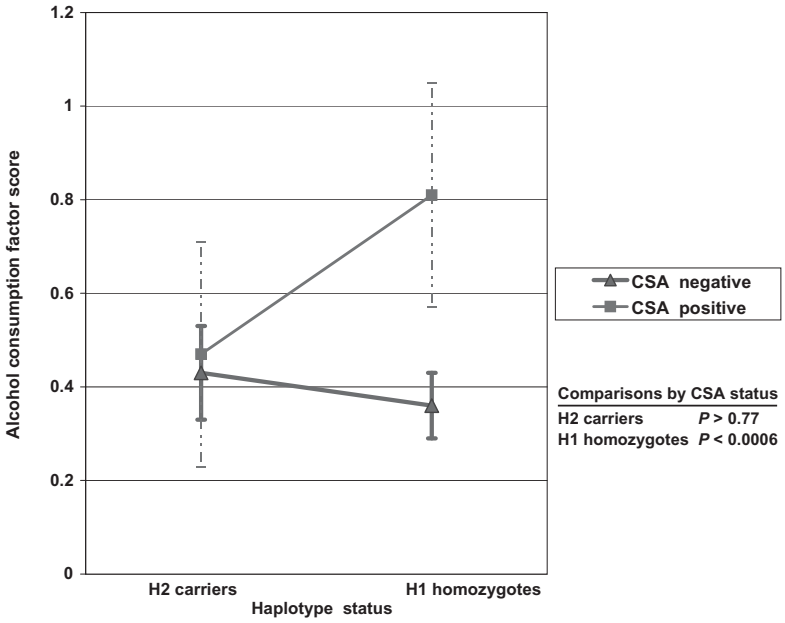

Figure 2 Mean alcohol consumption factor score by H1/H2 haplotype and childhood sexual abuse (CSA) status (error bars indicate 95% confidence intervals for each mean)

DISCUSSION

Our results provide evidence that CSA-associated risk for alcohol-related outcomes is moderated by the H2 haplotype. We first confirmed that a history of CSA is associated with significant risk for alcohol consumption and lifetime DSM-IV alcohol dependence in our sample; the H2 haplotype was not associated with risk for either of these outcomes. We found that, for both alcohol consumption and dependence, a G × E interaction involving CSA and the H2 haplotype is associated with significant protective effects. In those with the H2 haplotype, we observed no significant CSA-associated risk for either higher alcohol consumption or alcohol dependence.

Our results extend and clarify a prior report (Blomeyer *et al.* 2008) in which a significant G × E interaction involving severe stressors over the prior 3 years and the minor allele of rs1876831, a *CRHR1* SNP, led to protection against alcohol consumption in adolescents. We found evidence of a similar interaction and demonstrated that it involves the ~1.5 Mb H2 haplotype spanning several genes in this region of chromosome 17. rs1876831 is one of many informative markers that can be used to tag this haplotype. Another important feature that distinguishes our study from the prior report is that our larger, substantially older sample has already passed through the period of greatest risk for problematic alcohol use, enabling an examination of lifetime measures—peak ACFS and DSM-IV alcohol dependence diagnosis—that characterize respondents' mature drinking patterns. Both of these measures have excellent psychometric properties and have been shown to be at least moderately heritable (Bucholz *et al.* 1994; Heath *et al.* 1997; Agrawal *et al.* 2009; Grant *et al.* 2009). Our findings demonstrate that the protection against CSA-associated risk for problematic alcohol use associated with the H2 haplotype is persistent into adulthood. Overall, our results suggest that one or more of the five adjacent genes within the H2 haplotype play a major role in the risk for alcohol-related outcomes associated with severe life stressors.

Additional research will be necessary to clarify which gene or genes are responsible for these protective effects. Animal studies (Le *et al.* 2000; Shaham, Erb & Stewart 2000; Funk, Shaham & Lê *et al.* 2003; Hansson *et al.* 2006; Heilig & Koob 2007; Pastor *et al.* 2008; Sommer *et al.* 2008) published to date provide evidence that alterations in *CRHR1* expression are involved in important facets of alcohol consumption, dependence and relapse. Marchigian-Sardinian Preferring (msP) rats, selectively bred for high alcohol preference, have a *CRHR1* promoter polymorphism that results in increased *CRHR1* expression in limbic regions (Hansson *et al.* 2006). Alcohol self-administration by non-dependent msP rats is suppressed by CRF_1 antagonists; similar effects are not seen in unselected Wistar (control) rats. msP rats also display greater sensitivity to inhibition of foot-shock-induced ethanol reinstatement by a CRF_1 antagonist than do control rats. (Hansson *et al.* 2006). Rodents (not limited to those bred for ethanol preference) experience a prolonged period of increased anxiety and stress responsivity when withdrawn from ethanol after having been made dependent, symptoms that can be blocked by administration of a CRF_1 antagonist (Sommer *et al.* 2008). In these animals, up-regulation of *CRHR1* expression has been found in the basolateral and medial amygdalar nuclei

(Sommer *et al.* 2008) as have elevated corticotropin-releasing factor (CRF) levels in their central amygdalar nucleus. In rats previously dependent on ethanol (similar findings have been reported with heroin and cocaine) (Shaham *et al.* 2000), relapse can be induced by foot-shock stress. For each drug, this reinstatement can be blocked by intracerebroventricular infusion of CRF_1 antagonists (Le *et al.* 2000; Shaham *et al.* 2000). Direct CRF administration into involved brain regions also induces relapse in these animals (Le *et al.* 2000) and has been shown to block the increase in *c-fos* expression in the central nucleus of the amygdala that follows foot shock (Funk *et al.* 2003).

A recent report (Barr *et al.* 2009) provides evidence that findings from the rodent literature are also applicable to non-human primates. The authors describe a functional *CRH* promoter polymorphism in rhesus macaques that is associated with greater stress responsivity and report interactions between genotype and rearing condition with significantly greater adrenocorticotropic hormone (ACTH) and cortisol responses to social stress observed in peer-reared carriers of this polymorphism. Most importantly, they report a G × E interaction in which significantly greater alcohol consumption was found only for carriers of this polymorphism who had experienced early life stress (peer-rearing). This interaction, involving a *CRH* promoter polymorphism and a severe early life stressor associated with greater alcohol consumption in the absence of a significant main effect, is strikingly similar to the findings of the current report.

Other studies in rodents have demonstrated that exposure to severe, early life stressors results in epigenetically mediated alterations in gene expression (Weaver *et al.* 2004, 2005, 2007; Champagne *et al.* 2006). Although admittedly speculative at present, it is possible that the protective effect that we observed could result from one or more functional polymorphisms limiting CSA-related epigenetically mediated changes. Epigenetic modifications of gene expression due to ethanol are known to occur through a variety of different mechanisms (Pandey *et al.* 2008; Pietrzykowski *et al.* 2008; Shukla *et al.* 2008), and thus might not be susceptible to a similar protective effect (consistent with the lack of a significant main effect for H2 haplotype in our sample).

Our failure to confirm the earlier report (Treutlein *et al.* 2006) of a significant main effect for rs1876831 on lifetime prevalence of (any) binge drinking and drunkenness in their adolescent sample and on alcohol intake in clinically ascertained alcoholic adults may have resulted from a disparity in the two studies' outcome measures. The ACFS (Agrawal *et al.* 2009; Grant *et al.* 2009) used in this study is an estimate of consumption that is primarily based on the period of heaviest lifetime use. The main effects reported in the adolescent sample (Treutlein *et al.* 2006) were for two very early drinking career milestones, any episode of binge drinking or drunkenness. In their clinical sample, the significant main effect was for consumption of > 250 g of ethanol daily prior to admission, a binary measure of daily consumption much later in the course of the disorder that could be affected by various covariates (e.g. gender, *ADH* genotype, alcoholic liver disease). Another study (Dahl *et al.* 2005) that examined whether *CRHR1* polymorphisms were associated with alcohol dependence risk in European American alcoholics found no association for any SNPs, including those informative for the H2 haplotype.

Several additional issues should be considered when interpreting our results. We substituted CSA for the measure of multiple severe stressors in adolescence used in the prior report (Blomeyer *et al.* 2008). Previous reports of G × E interactions have included either multiple classes of stressors (Caspi *et al.* 2003) or substituted stressors for which data were available in replication studies (Kaufman *et al.* 2004; Gillespie *et al.* 2005; Kendler *et al.* 2005; Surtees *et al.* 2006). Additional study will be necessary to delineate whether our findings will generalize to different types of stressors or those occurring during other developmental periods. CSA is also associated (Nelson *et al.* 2002) with risk for subsequent stressors further complicating definitive attribution. Although use of binary outcome variables for examination of G × E interactions may predispose to spurious positive findings (Eaves 2006), the association we observed with ACFS, a continuous measure, is less susceptible to this source of error. Given concerns about the sensitivity of inferences from G × E interactions to scaling, we ran an additional analysis using Huber robust regression, which down-weights outlier observations: the G × E interaction term remained significant ($P = 0.026$) with a modest reduction in effect size (beta = −0.37 versus beta = −0.41 in the linear regression analyses). The association with alcohol-related phenotypes could be bidirectional; however, the mean age (11.0 years) of first CSA occurrence suggests that CSA typically precedes alcohol problems. The use of a binary variable for CSA combines diverse abuse experiences encompassing a wide range of severity; a stronger association might have been observed if a quantitative covariate incorporating information on abuse severity and duration were available. Retrospective assessment of CSA in adults may raise concerns regarding the introduction of bias. The CSA measure used in this study has been found to have reasonable concordance within female like-sex twin pairs and significant association with psychiatric sequelae and parental risk factors (Dinwiddie *et al.* 2000; McLaughlin *et al.* 2000). Examination of discordant pairs found no evidence for retrospective bias in the association of CSA with parental rejection (McLaughlin *et al.* 2000). The

prevalence of CSA in our sample is higher than is typical for community samples, a likely result of enrichment for heavy smoking. Because of this enrichment, additional studies will be necessary to determine whether our results will generalize to samples representative of the general population. *Post hoc* analyses of genetically informative data on ancestry from a partially overlapping genome-wide association study (GWAS) revealed that two families ($n = 4$ individuals in total) are outliers on the basis of ancestry. The ACFS of these families does not differ significantly from the remainder of the sample; dropping these four individuals has very minimal effect on either interaction term point estimates or *P*-values.

Our data suggest that the H2 haplotype is protective against CSA-associated risk for higher lifetime alcohol consumption and alcohol dependence. The extent of these protective effects suggests that one or more of the genes within the H2 haplotype are playing an important role in stress-associated risk for alcohol consumption and dependence. Although the evidence (Le *et al.* 2000; Hansson *et al.* 2006; Heilig & Koob 2007; Pastor *et al.* 2008; Sommer *et al.* 2008) from animal research is quite strong, it is premature to conclude that these protective effects are due to a *CRHR1* polymorphism. Additional research is needed to determine definitively the gene or genes responsible for these protective effects. Gene expression studies have provided strong evidence in favor of *MAPT* (Caffrey *et al.* 2006; Caffrey, Joachim & Wade-Martins 2008) versus *CRHR1* (Campdelacreu *et al.* 2006) involvement in PSP risk, although other research (Cruchaga *et al.* 2009) suggests that multiple genes may be involved. However, since our finding involves a G × E interaction rather than a main effect, it will be considerably more difficult to conduct this type of investigation. If the effects that we observe are a consequence of a *CRHR1* polymorphism, our results may have immediate clinical relevance. Researchers have recently developed (Gehlert *et al.* 2007) improved CRF_1 antagonist drugs that are already scheduled for pharmaceutical company-sponsored clinical trials of alcohol dependence treatment. Our findings would predict that H1 homozygotes with a history of severe early trauma exposure will preferentially respond to these agents. More generally, our findings emphasize the potential utility of screening for severe early trauma exposure in individuals presenting for alcohol dependence treatment.

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Authors Contribution

PM, JCW, and EN were responsible for the study concept and design. AA, MP, JCW, JBW, JK, GM, AH, NGM, and PAFM contributed to the acquisition of data. EN, AA, MP, FS, JG, AS, JR, AH, and AG assisted with data analysis and interpretation of findings. EN drafted the manuscript. AA, MP, JCW, JBW, FS, JK, JG, AS, JR, GM, AH, AG, NM, and PM provided critical revision of the manuscript for important intellectual content. AA, JBW, AH, NM, PM obtained funding for the project. MP, JK, GM, NM, and PM provided administrative, technical, or material support. JCW, GM, AG, NM, and PM supervised the study. All authors critically reviewed content and approved final version submitted for publication.

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