Interacting effects of CRHR1 gene and stressful life events on drinking initiation and progression among 19-year-olds

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Abstract

Research in animals and first results in adolescents have indicated that genetic variation in the corticotropin-releasing hormone receptor 1 (CRHR1) is associated with heavy alcohol consumption related to stress. The purpose of this study was to determine whether two haplotype-tagging single nucleotide polymorphisms covering the CRHR1 gene (rs242938, rs1876831) interact with stressful life events affecting age at drinking initiation and alcohol consumption in young adults. Participants were drawn from the Mannheim Study of Children at Risk, an epidemiological cohort study following the outcome of early risk factors. Structured interviews were administered to 270 participants (125 males, 145 females) at 15 yr and 19 yr to assess age at first drinking and, at 19 yr, to assess current drinking and recent stressful life events. Life events during childhood and child psychopathology were measured using standardized parent interviews. Results indicated that, even after control for a range of confounders, higher numbers of stressful life events prior to drinking onset were significantly related to earlier age at first drink only among homozygotes for the C allele of rs1876831. Earlier age at drinking onset was significantly associated with higher consumption levels in 19-yr-olds. Furthermore, homozygotes of the rs1876831 C allele as well as carriers of the rs242938 A allele, when exposed to stress, exhibited significantly higher drinking activity than carriers of other alleles. These findings extend previous reports by demonstrating that the CRHR1 gene and stressful life events interact to predict both drinking initiation in adolescence and progression of heavy alcohol use in young adulthood.

Key words: Alcoholism, corticotropin-releasing hormone receptor 1, gene × environment interaction, stressful life events.

Introduction

While there is ample evidence that early drinking onset is a risk factor for high levels of alcohol consumption and alcohol dependence in adulthood (Grant et al. 2001a; Grant & Dawson, 1997), the mechanisms underlying this relationship are still being discussed.

In addition to childhood externalizing disorders, parental substance use and peer models (Donovan, 2004), stressful experiences have been associated with an earlier age of drinking onset in several cross-sectional studies (Zimmermann et al. 2007). In a retrospective cohort study of 8000 adults, adverse childhood experiences were found to be strongly related to drinking initiation in early and mid-adolescence (Dube et al. 2006). Other studies demonstrated daily problems and life events as predictors of the age of drinking initiation (Sartor et al. 2007). Although twin studies suggest that drinking initiation is determined more
by environmental influences than the establishment of drinking patterns (Rose et al., 2004), a heritable component of early drinking onset has also been postulated (McGue et al., 2001).

One possible way to explain how this genetic vulnerability manifests itself might be the individual’s response to stress. In particular, stressful experiences and the body’s response to stressful events may play an important role in the progression of alcohol use (Veenstra et al., 2006). The stress-coping model of substance use (Wills & Filer, 1996) has been postulated to explain the relationship between stressful events and alcohol consumption, with alcohol being used to regulate negative affect. However, empirical evidence concerning the impact of life events on alcohol consumption is heterogeneous, indicating that the association between stress and alcohol use is different depending on categories of life events (Dawson et al., 2005), gender (Rutledge & Sher, 2001) and age (Aseltine & Gore, 2000). Considering the fact that the influence of stress on drinking is not fully attributable to coping and affect regulation (Park et al., 2004), a moderating role of a genetic vulnerability in the stress system has been suggested.

Stress activates the corticotropin-releasing hormone (CRH) system, resulting in the hypersecretion of glucocorticoids from the adrenal gland. In addition, the extrahypothalamic component of the CRH system stimulates the amygdaloid CRH system. Animal research has demonstrated that activation of the extrahypothalamic CRH system results in many of the behavioural consequences observed following stress (Makino et al., 1999). The activation of CRH receptors in the central nucleus of the amygdala (CEA) and/or CRH pathways emanating from the CEA plays an important role in fear-related behaviours (Makino et al., 2002). In particular, CRHR1 is known to mediate behavioural stress responses (Heinrichs & Koob, 2004). Receptors for CRH belong to the seven-transmembrane domain, G protein-coupled receptor family and signal through the cyclic AMP pathway (Aguilera, 1994). Two different CRH receptors have been isolated, which share 70% sequence identity (OMIM ID *122561) and are designated types 1 and 2, CRHR1 and CRHR2, respectively. Each of the two receptors is encoded by a separate gene. The human CRHR1 RefSeq (NM_004382) maps on chromosome 17q21.31 (chr17: 41217449-41268973) and contains 13 exons.

Alcohol is a potent anti-anxiety agent. It has been shown that elevated anxiety predicts alcohol use in rats (Spanagel et al., 1995), whereas involvement of CRH transmission in the effects of stress on alcohol drinking has been demonstrated in non-functional CRHR1 mouse mutants (Sillaber et al., 2002). In the genetically selected alcohol-preferring msP rat line, an innate up-regulation of the CRHR1 transcript encoding CRHR1 was observed in several limbic brain areas related to alcohol-drinking motivation. This up-regulation was associated with a genetic polymorphism of the CRHR1 promoter and was accompanied by increased CRHR1 density (Hansson et al., 2006). Pharmacological experiments with the CRHR1 selective antagonist antalarmin have indicated that up-regulated CRHR1 transmission in msP rats drives excessive alcohol intake and stress-induced reinstatement of alcohol-seeking following extinction. Further animal studies demonstrated that voluntary alcohol consumption was able to down-regulate CRHR1 transcript levels in msP rats in brain areas like the central and medial amygdala, where elevated expression has been detected previously (Hansson et al., 2007). As there were no differences in alcohol-seeking between the msP line and Wistar rats after extinction and in the absence of an environmental stressor, the effect of genetic variation at the CRHR1 locus represents a prototypical gene × environment (G × E) interaction (Hansson et al., 2006).

In humans, two haplotype-tagging single nucleotide polymorphisms (SNP, rs242938 and rs1876831, see Fig. 1) of CRHR1 have recently been associated with heavy drinking behaviour in adolescents (Treutlein et al., 2006). Of these, one (rs1876831) is represented in the HapMap project database (http://www.hapmap.org/), which allows pair-wise linkage disequilibrium (LD) for this marker to be assessed on a genome-wide scale. SNAP Proxy Search (Johnson et al., 2008) on phased HapMap data revealed that the tagging ability of rs1876831 exceeds that computed from the limited initial dataset of 14 markers. Markers tagged by rs1876831 ($r^2 \geq 0.8$) are distributed in a region of >780 kb (chr17: 40931011-41713128) on chromosome 17, but not all markers in this chromosomal region are in $r^2 \geq 0.8$ with rs1876831. For example, significantly associated markers in two recent publications reporting the influence of this gene (Bradley et al., 2008; Wasserman et al., 2008) were not correlated strongly enough to rs1876831 ($r^2 = 0.18$; rs110402–rs1876831: $r^2 = 0.204$; rs7209436–rs1876831: $r^2 = 0.197$) to compare across studies. The large size of the region, in which polymorphisms are tagged by rs1876831 is compatible with the long-range LD described for this chromosomal region (Tantisira et al., 2008). The second tagging SNP, rs242938, has no HapMap entry, so that its correlation with significantly associated markers of other studies cannot be analysed. Hypotheses on the
functional role of this locus suggest that rs1876831 alters an intronic binding site for transcription factor Sp1, which may contribute to intronic enhancers or silencers and lead to a genotype-specific transcriptional activation resulting in differential amounts of available CRHR1. However, given the large number of SNPs tagged by the selected SNPs, it is unclear, which of these or which combination of these SNPs are responsible for an observed association.

In a subsequent study, first evidence was provided in humans that genetic variation in CRHR1 moderates the impact of stress on heavy drinking in adolescents (Blomeyer et al. 2008). In 15-yr-olds, the number of stressful life events during the past 3 yr was found to be significantly related to increasing rates of heavy drinking only among individuals homozygous for the C allele of the haplotype-tagging SNP rs1876831. In order to extend and further explore these findings, we used a prospective longitudinal design to test the hypotheses that (1) the interaction between preceding lifetime stressors and the CRHR1 gene predicts age at drinking onset in adolescence and (2), in addition to the effect of early drinking onset, the interaction between recent life events and genetic vulnerability in the body’s response to stress continues to predict higher alcohol consumption levels in early adulthood.

**Method**

**Participants**

Participants were drawn from the Mannheim Study of Children at Risk, a prospective longitudinal study, following the development of at-risk children from infancy into adulthood (Laucht et al. 2000). The initial sample comprised 384 children of predominantly (>99.0%) European descent born between 1986 and 1988. Infants were recruited from two obstetric and six children’s hospitals of the Rhine-Neckar Region of Germany and were included consecutively into the sample according to a two-factorial design intended to enrich and control the risk status of the sample (for more details see Laucht et al. 1997). As a result, approximately one third of the study sample had experienced severe obstetric complications such as preterm birth, while about one third of the families suffered from severe psychosocial adversities such as broken home history of a parent or psychiatric disorder of a parent. To control for confounding effects of family environment and infant medical status, only firstborn children with singleton birth, German-speaking parents, and no severe physical handicaps, obvious genetic defects or metabolic diseases were included. Assessments were conducted at ages: 3 months, 2, 4.5, 8, 11, 15 yr, and most recently at age 19 yr. Of the initial sample of 384 participants, 18 (4.7%) were excluded because of severe handicaps (IQ or MQ <70 or neurological disorder), 37 (9.6%) were drop-outs, 35 (9.1%) refused to participate in blood sampling and 24 had incomplete data (6.3%). The current investigation included 270 young adults (125 males, 145 females) who participated in the 19-yr assessment and for whom data on CRHR1 genotypes, stressful life events and alcohol consumption were complete. Loss of participants was not selective with regard to sex, but was selective in terms of risk group,
with drop-outs suffering from more psychosocial adversities \((p < 0.01)\). The excluded probands did not differ from the included participants in terms of age at drinking onset and drinking behaviour. The study was approved by the ethics committee of the University of Heidelberg and written informed consent was obtained from all participants.

**Assessment**

At the ages of 15 and 19 yr, participants were administered a drinking inventory including *lifetime alcohol use* and *age at drinking onset*, defined as having drunk a standard drink (8–12 g alcohol) for the first time. The inventory is part of the Substance Use Questionnaire (SUQ) designed by Müller & Abbet (1991) in collaboration with the World Health Organization (WHO). If participants drank their first alcoholic beverage before 15 yr, information about age at drinking onset was gathered at age 15 yr; otherwise the information given at 19 yr was used in order to reduce false-memory effects. The Alcohol Timeline Followback (TLFB; Sobell & Sobell, 2003) method was used to assess *drinking behaviour* at 19 yr, providing estimates of the distribution of drinking days and the amount of daily alcohol consumption in the last 45 d. Four drinking variables were derived, indexing the overall number of standard drinks consumed during the last 45 d, the maximum number of drinks consumed per occasion, the frequency of drinking days, and the frequency of heavy-drinking days, which were defined as having drunk five (females: four) or more standard drinks in a row. Good reliability for assessing current drinking with this instrument has been reported (Sobell et al. 1988).

To measure the exposure to *stressful life events during childhood and early adolescence*, a semi-structured interview was administered to the parents when the child was aged 2–15 yr. At the age of 19 yr, the interview was conducted with the young adults to assess the exposure to stressful life events *during late adolescence* (period of the past 4 yr). The interview, a modified and shortened version of the Munich Events List (MEL; Maier-Diewald et al. 1983), assessed the occurrence of stressful life events and chronic difficulties during the period of years until the last assessment. The 28–53 age-specific items covered all relevant areas of children’s, adolescents’ and young adults’ life stress, including family (divorce/separation, pregnancy), school (change, suspension), parents (legal problems, job loss), health (serious illness/injury), and living conditions (major financial problems). Several indices can be derived from the MEL, such as total index, weighted total and chronic life events. For the current analysis, a total life-event score was computed which counted the number of life events present (2 yr: \(\text{mean} = 5.44, \text{S.D.} = 3.06, \text{range} \ 0–21\); 4.5 yr: \(\text{mean} = 7.02, \text{S.D.} = 3.44, \text{range} \ 0–21\); 8 yr: \(\text{mean} = 6.54, \text{S.D.} = 3.48, \text{range} \ 0–21\); 11 yr: \(\text{mean} = 5.43, \text{S.D.} = 3.41, \text{range} \ 0–18\); 15 yr: \(\text{mean} = 3.54, \text{S.D.} = 2.23, \text{range} \ 0–11\); 19 yr: \(\text{mean} = 2.82, \text{S.D.} = 2.64, \text{range} \ 0–14\). The \(z\)-standardized scores of the life events from each assessment prior to drinking onset were summed up, indexing total life stress during the period before drinking began. The reliability and validity of the MEL has been confirmed in several studies (Wittchen et al. 1989).

*Externalizing symptoms* were assessed with the Mannheim Parent Interview (MEI; Esser et al. 1989) at age 2, 4, 8 and 11 yr and with the Schedule for Affective Disorders and Schizophrenia for School-Aged Children (K-SADS-PL; German version by Delmo et al. 2000) at age 15 yr. The MEI is a highly structured interview adapted from Rutter’s parent interviews (Cox & Rutter, 1985), which was modified to include all symptoms related to major DSM-IV diagnoses. The K-SADS is a widely used structured diagnostic interviews completed independently with parents and adolescents, for which a considerable body of reliability and validity data has been published (Kaufman et al. 2008). The number of DSM-IV ADHD, CD and ODD symptoms present was calculated for each assessment, \(z\)-standardized and sum scores were formed, indexing severity of externalizing symptoms prior to drinking onset.

*Psychosocial risk factors* were determined according to an ‘enriched’ family adversity index as proposed by Rutter & Quinton (1977) at the 3-month assessment measuring the presence of 11 adverse family factors covering characteristics of the parents (e.g. broken home history), the partnership (e.g. marital discord), and the family environment (e.g. overcrowding) during a period of 1 yr prior to birth. After a screening for substance use problems, a standardized clinical interview (SCID, German version by Wittchen et al. 1997) was administered to the parents at each assessment to obtain prevalence and lifetime *diagnosis of alcohol abuse and dependence* in parents. In the case of absent fathers, evaluation of paternal alcohol use relied on maternal report.

**Tagging SNP selection and genotyping**

Among the genetic variations of the CRHRI gene in public databases, 14 SNPs were selected and genotyped in a group of \(n = 150\) healthy individuals. These 14 SNPs were systematically screened for their LD
structure, their redundancy was analysed and tagging SNPs which minimize the loss of information were chosen. Despite the fact that the dataset contained 14 markers, subsequent LD analyses of these markers revealed that a minimal set of two tagging SNPs was sufficient to distinguish the common haplotypes of this region (Treutlein et al. 2006). The further analyses concentrated on the two tagging SNPs, rs242938 and rs1876831, which are in pairwise LD ($\phi \geq 0.8$) with the remaining 12 markers.

Genomic DNA was isolated from whole blood with the Qiap kit (Qiagen, USA) and genotyped for the two tagging SNPs rs242938 and rs1876831 of CRHR1. For polymerase chain reaction (PCR), HotStar Taq-DNA Polymerase (Qiagen) was used under standard cycling conditions with 4 ng template DNA in a total volume of 25 $\mu$L PCR reaction. Best oligonucleotide pairs were selected from the flanking sequences provided by SNP databases. Genotyping was carried out with restriction fragment length polymorphism (RFLP) for both polymorphisms and replicated for 15% of the samples with different experimental analyses to ensure consistency of results across genotyping methods: the TaqMan genotyping assay procedure was used to replicate rs242938 (100% identical), and direct sequencing to replicate rs1876831 (100% identical). Genotype frequencies were 84.3% (GG), 14.6% (GA) and 1.1% (AA) for rs242938, and 64.6% (CC), 30.7% (CT) and 4.6% (TT) for rs1876831, respectively. Distribution of genotypes did not deviate from Hardy–Weinberg equilibrium.

Data analysis

Hierarchical Cox regression analyses with time-dependent covariates were used to predict age at drinking onset. Time-dependent variables were the number of stressful life events and externalizing symptoms experienced from age 2 yr until the last assessment before drinking onset. To examine G × E effects on drinking measures, hierarchical linear regressions were computed. All models were fitted for the main effects of the rs242938 and rs1876831 genotypes, respectively, and for the main effect of stressful life events with subsequent addition of the interaction term. Models included sex, psychosocial risk factors, externalizing symptoms and both parents’ lifetime history of alcohol abuse or dependence as covariates. When predicting drinking behaviour at age 19 yr, age at drinking onset was introduced as a further covariate. The total number of life events was z-standardized for better interpretation of potential main effects in the case of a significant interaction. For these analyses, genotypes were dichotomized according to homozygosity for the major alleles (rs242938: G allele; rs1876831: C allele).

Results

Descriptive data for drinking variables, stressful life events and covariates (sex, psychosocial adversity, childhood externalizing symptoms, and parental alcohol use disorders, $n=47$) in the total sample, and separately for each genotype, are presented in Table 1. Genotype groups did not differ significantly with regard to any of these measures, except for age at drinking onset, with a significantly younger age at first drink among carriers of the CC genotype of rs1876831 compared to T allele carriers. Furthermore, there were no significant differences between genotype groups with regard to stressful life events. In addition, no significant differences according to sex were observed on any of these variables.

The findings of Cox regression models testing for the effect of CRHR1 genotype, stressful life events and their interaction on age at drinking initiation are summarized in Table 2. Among covariates, gender, psychosocial adversity, and externalizing symptoms were related to drinking onset. Genotype group and the number of stressful life events experienced in the lifetime before drinking onset did not predict age at drinking initiation. However, a significant interaction between CRHR1 genotype and stress exposure was obtained for rs1876831 but not for rs242938. Figure 2 illustrates the interaction, demonstrating that more stressful life events during the time period before drinking onset were associated with earlier drinking initiation among CC individuals, but not among CT/TT individuals.

The results of linear regression models testing for the impact of CRHR1 genotype and stressful life events during adolescence (15–19 yr) on young adult drinking behaviour are summarized in Tables 3 and 4. Among covariates, gender was associated with all drinking measures, while childhood externalizing symptoms were related to drinking days. Age at drinking onset had a significant effect on all measures of drinking behaviour except for the maximum number of standard drinks consumed per occasion, where it was only a trend. The earlier the participants started drinking, the more alcohol they consumed at age 19 yr.

Concerning rs1876831, a significant interaction between CRHR1 genotype and stress exposure on the total number of standard drinks consumed as well as a trend for the number of binge-drinking days emerged.
Table 1. Drinking behaviour, stressful life events and relevant covariates depending on CRHR1 genotypes

<table>
<thead>
<tr>
<th>rs1876831 genotype</th>
<th>rs242938 genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC (n = 175)</td>
<td>CT/TT (n = 95)</td>
</tr>
<tr>
<td><strong>Age (yr) at first drink</strong> (n = 268)</td>
<td>13.7 (1.6)</td>
</tr>
<tr>
<td><strong>Number of standard drinks</strong> (last 45 d)</td>
<td>31.3 (46.1)</td>
</tr>
<tr>
<td><strong>Maximum number of standard drinks/occasion</strong></td>
<td>6.8 (8.1)</td>
</tr>
<tr>
<td><strong>Drinking days</strong> (last 45 d)</td>
<td>7.2 (6.9)</td>
</tr>
<tr>
<td><strong>Binge-drinking days</strong> (last 45 d)</td>
<td>2.5 (3.9)</td>
</tr>
<tr>
<td><strong>Childhood stressful life events</strong> (2–8 yr)</td>
<td>-0.04 (2.3)</td>
</tr>
<tr>
<td><strong>Adolescence stressful life events</strong> (15–19 yr)</td>
<td>3.0 (2.7)</td>
</tr>
</tbody>
</table>

**Covariates**

| Sex (male), n (%) | 83 (47.4) | 42 (44.2) | 102 (44.7) | 23 (54.8) | 125 (46.3) |
| Psychosocial adversity | 2.0 (2.0) | 1.9 (2.2) | 2.0 (2.1) | 1.8 (1.8) | 2.0 (2.1) |
| Childhood externalizing symptoms | 0.08 (2.5) | -0.18 (2.2) | -0.04 (2.4) | 0.12 (2.2) | -0.01 (2.4) |
| Parental lifetime alcohol use disorders, n (%) | 35 (20.0) | 12 (12.6) | 40 (17.5) | 7 (16.7) | 47 (17.4) |

Values given are means (s.d.).

* Defined as drinking > 5 (females: 4) standard drinks (each with 8–12 g alcohol) in a row.

** z-standardized number of life events, summed up over three assessments (2, 4, 8 yr).

| Table 2. Cox regression models testing the effects of CRHRI genotype, stressful life events prior to drinking onset, and their interaction on age at first drink (n = 270) |

<table>
<thead>
<tr>
<th>Predictors</th>
<th>Age at first drink (yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
</tr>
<tr>
<td>Sex</td>
<td>0.73</td>
</tr>
<tr>
<td>Psychosocial adversity</td>
<td>0.92</td>
</tr>
<tr>
<td>Childhood externalizing symptoms</td>
<td>1.09</td>
</tr>
<tr>
<td>Parental lifetime alcohol use disorders</td>
<td>1.10</td>
</tr>
<tr>
<td>rs1876831 genotype</td>
<td>1.15</td>
</tr>
<tr>
<td>Stressful life events*</td>
<td>0.96</td>
</tr>
<tr>
<td>rs1876831 × stressful life events</td>
<td>1.10</td>
</tr>
<tr>
<td>Sex</td>
<td>0.75</td>
</tr>
<tr>
<td>Psychosocial adversity</td>
<td>0.93</td>
</tr>
<tr>
<td>Childhood externalizing symptoms</td>
<td>1.08</td>
</tr>
<tr>
<td>Parental lifetime alcohol use disorders</td>
<td>1.12</td>
</tr>
<tr>
<td>rs242938 genotype</td>
<td>0.95</td>
</tr>
<tr>
<td>Stressful life events*</td>
<td>1.01</td>
</tr>
<tr>
<td>rs242938 × stressful life events</td>
<td>1.04</td>
</tr>
</tbody>
</table>

HR, Hazard ratio; CI, confidence interval.

* Time-dependent variable, number of stressful life events from 2 yr until drinking onset.

Fig. 2. Age at first drink grouped by CRHRI rs1876831 genotype and exposure to stressful life events prior to drinking onset (2–8 yr) (adjusted for sex, psychosocial risk factors, externalizing symptoms in childhood and parents’ lifetime alcohol use disorders). The exact illustration of Cox regression results using time-dependent covariates is not feasible. As 8 yr was the earliest age at first drink among the participants, the interaction with the life events of the time period between 2 yr and 8 yr was depicted for demonstration purposes, thus ensuring that exposure to life events preceded drinking onset in all cases. □, < Median; ■, ≥ median.
Table 3. Linear regression models testing the effects of the CRHR1 rs1876831 genotype, stressful life events during adolescence, and their interaction on drinking behaviour in 19-yr-olds (n = 268)

<table>
<thead>
<tr>
<th>Predictors</th>
<th>No. of standard drinks</th>
<th>Maximum no. of standard drinks/occasion</th>
<th>Drinking days</th>
<th>Binge-drinking days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>p</td>
<td>β</td>
<td>p</td>
</tr>
<tr>
<td>Sex</td>
<td>−0.335</td>
<td>&lt;0.001</td>
<td>−0.367</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Psychosocial adversity</td>
<td>−0.032</td>
<td>0.606</td>
<td>0.055</td>
<td>0.378</td>
</tr>
<tr>
<td>Childhood externalizing symptoms</td>
<td>−0.039</td>
<td>0.530</td>
<td>−0.057</td>
<td>0.373</td>
</tr>
<tr>
<td>Parental lifetime alcohol use disorders</td>
<td>−0.011</td>
<td>0.845</td>
<td>−0.008</td>
<td>0.887</td>
</tr>
<tr>
<td>Age at first drink</td>
<td>−0.120</td>
<td>0.033</td>
<td>−0.098</td>
<td>0.087</td>
</tr>
<tr>
<td>rs1876831 genotype</td>
<td>−0.057</td>
<td>0.303</td>
<td>−0.020</td>
<td>0.727</td>
</tr>
<tr>
<td>Stressful life events*</td>
<td>0.695</td>
<td>&lt;0.001</td>
<td>0.418</td>
<td>0.014</td>
</tr>
<tr>
<td>rs1876831 × stressful life events</td>
<td>−0.363</td>
<td>0.028</td>
<td>−0.124</td>
<td>0.462</td>
</tr>
</tbody>
</table>

*Number of stressful life events between 15 yr and 19 yr.

Table 4. Linear regression models testing the effects of the CRHR1 rs242938 genotype, stressful life events during adolescence, and their interaction on drinking behaviour in 19-yr-olds (n = 268)

<table>
<thead>
<tr>
<th>Predictors</th>
<th>No. of standard drinks</th>
<th>Maximum no. of standard drinks/occasion</th>
<th>Drinking days</th>
<th>Binge-drinking days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>p</td>
<td>β</td>
<td>p</td>
</tr>
<tr>
<td>Sex</td>
<td>−0.341</td>
<td>&lt;0.001</td>
<td>−0.366</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Psychosocial adversity</td>
<td>−0.033</td>
<td>0.590</td>
<td>0.056</td>
<td>0.366</td>
</tr>
<tr>
<td>Childhood externalizing symptoms</td>
<td>−0.028</td>
<td>0.653</td>
<td>−0.050</td>
<td>0.431</td>
</tr>
<tr>
<td>Parental lifetime alcohol use disorders</td>
<td>−0.002</td>
<td>0.975</td>
<td>−0.004</td>
<td>0.938</td>
</tr>
<tr>
<td>Age at first drink</td>
<td>−0.153</td>
<td>0.006</td>
<td>−0.114</td>
<td>0.045</td>
</tr>
<tr>
<td>rs242938 genotype</td>
<td>0.034</td>
<td>0.530</td>
<td>0.040</td>
<td>0.464</td>
</tr>
<tr>
<td>Stressful life events*</td>
<td>−0.248</td>
<td>0.159</td>
<td>0.000</td>
<td>&gt;0.999</td>
</tr>
<tr>
<td>rs242938 × stressful life events</td>
<td>0.626</td>
<td>&lt;0.001</td>
<td>0.311</td>
<td>0.080</td>
</tr>
</tbody>
</table>

*Number of stressful life events between 15 yr and 19 yr.

(Table 3). Subsequent analysis demonstrated that higher numbers of stressful life events were associated with a higher amount of drinking and an increasing number of binge-drinking days, respectively, among CC individuals (β = 0.429, p < 0.001; β = 0.445, p < 0.001, respectively), but not among CT/TT individuals (β = 0.118, p = 0.301; β = 0.137, p = 0.242, respectively). Figure 3 illustrates the interaction for the total number of drinks. Interactions between rs1876831 and life stress with regard to the maximum number of standard drinks consumed per occasion and the number of drinking days were not observed. Furthermore, there was a significant main effect of life events on all drinking measures except drinking frequency. In contrast to previous analyses with the same sample concerning drinking behaviour at age 15 yr (Blomeyer et al. 2008), no significant main effect of rs1876831 on drinking variables was found.

Results were similar for rs242938, with significant interactions between stress exposure and genotype.
on the total amount of alcohol and the number of binge-drinking days (Table 4). Two subsequent separate regression analyses revealed that the association between stress exposure and alcohol consumption was present for both genotype variants, but was more than twice as high in GA/AA individuals ($\beta = 0.685, p < 0.001; \beta = 0.707, p < 0.001$, respectively) than in GG individuals ($\beta = 0.262, p < 0.001; \beta = 0.274, p < 0.001$, respectively). Figure 4 demonstrates the interaction for the number of drinks, using a median split on the life-events measure. After controlling for the interaction term, neither a main effect of genotype nor of life events on consumption measures was found for rs242938. There were no significant two-way interactions between the two tagging SNPs or three-way interactions with these markers and stress exposure on any of the drinking measures (data not shown, all $p > 0.40$).

Discussion

Using longitudinal data from an epidemiological cohort sample, this study adds to previous research, demonstrating that genetic variation in CRHR1 is associated with heavy alcohol consumption following repeated stress. The contribution of the present study is twofold. First, our findings provide evidence that CRHR1 moderates the impact of childhood stress exposure on age at drinking onset, an important predictor of later alcohol dependence. Even after controlling for a range of confounders, more stressful life events since age 2 yr up to the first drink were significantly related to earlier drinking initiation only among homozygotes for the C allele of rs1876831. These individuals had their first drink on average 7 months earlier when the median split on the number of life events up to 8 yr was considered. In contrast, variation in rs242938 was unrelated to alcohol initiation.

Second, the present investigation demonstrates significance of the interaction between CRHR1 and adolescent stress exposure on young adult consumption levels, revealing an effect of stressful life events on heavy drinking only among individuals homozygous for the C allele of rs1876831 as well as among individuals carrying the A allele of rs242938. Controlling for major confounding variables of drinking onset and progression, such as externalizing symptoms, parental alcohol use disorders, psychosocial adversity and gender, eliminated strong environmental as well as genetic contributions of these factors and ascertained that the interaction effects were not only byproduct of these covariates.

Because the C allele is the more frequent major allele of rs1876831, with a frequency of 80% in our sample, these results can be interpreted as suggesting...
a protective effect of the minor T allele. Carriers of this allele were found to have started drinking later and to drink less alcohol when exposed to stress at 19 yr. In contrast, the A allele of rs242938, with a frequency of 8% in our sample, has to be interpreted as a risk factor for heavy drinking in response to stress.

The findings reported above are in accordance with previous results from the Mannheim Study of Children at Risk (Blomeyer et al. 2008), revealing a G × E interaction between CRHR1 genotype and stressful life events on alcohol use in 15-yr-olds, with homozygous carriers of the rs1876831 A allele exhibiting higher drinking activity when exposed to stress than carriers of the G allele. Confirming and extending these findings, the present investigation indicated that this G × E interaction (i) even held when controlling for a number of major confounders, and (ii) persisted when observed during transition into adulthood. Moreover, the current findings extended the scope of this G × E interaction by demonstrating association with the phenotype of age at drinking onset. During the past two decades, a burgeoning literature has emerged establishing an association between age at drinking onset and risk for later hazardous alcohol consumption and dependence. Both cross-sectional (Hingson et al. 2006) and longitudinal studies (Grant et al. 2001b) have indicated that age at drinking onset is one of the strongest predictors for the development of later alcohol-related problems. The finding that childhood stress exposure in interaction with the CRHR1 genotype probably acts as preparation for this may be considered a significant contribution to unravelling the mechanisms underlying this association. This finding is of particular importance as, due to the prospective study design and the statistical procedure used, we were able to confirm that stress exposure preceded drinking onset.

Finally, in contrast to Blomeyer et al. (2008), the present investigation provided evidence for G × E interaction on alcohol consumption with regard to rs242938. The finding that the rs242938 × stress interaction is not associated with adolescent but with young adult drinking may be interpreted as suggesting an age-specific effect of this genetic variation, emerging only with the progression of alcohol consumption and the further establishment of the drinking-tocope motive (Park & Levenson, 2002). Behavioural genetics studies have strongly indicated the presence of genetic factors for multiple aspects of drinking. Moreover, these studies demonstrated that the influence of genetic factors may change in the progression of alcohol use from experimentation towards dependence (Hopfer et al. 2003). The transition from adolescence to adulthood may be viewed as a developmental period in which individual differences in drinking behaviour and drinking motives become particularly salient (Perkins, 1999; Rutledge & Sher, 2001). However, the majority of association studies conducted so far have neglected a developmental perspective, using subjects whose ages spanned a wide range.

Based on the findings reported above, this study suggests a double effect of the stress × CRHR1 genotype interaction. While rs1876831 seems to play a role in drinking initiation and heavy drinking during adolescence, rs242938 obviously becomes more important in the progression of stress-related drinking in early adulthood. As the interaction of rs1876831 with life events on alcohol consumption at age 19 yr remained statistically significant even after controlling for age at drinking onset, this effect cannot be traced back to the similar interaction between life events and rs1876831 already shown for age at drinking onset. The fact that the interaction between stress exposure and CRHR1 genotype is not only restricted to consumption levels but also predicts drinking initiation rules out the possibility that the genetic effect can be entirely explained by the different response to alcohol consumption. Genetic vulnerability rather has to take into account a more general disposition to deal with stress. However, it remains unclear whether individuals carrying the risk genotype are generally more sensitive to stress or whether they only use alcohol more intensely in order to cope with it. Moreover, a combination of these hypotheses is also reasonable, as reported by Cooper et al. (1995), who indicated that drinking to cope mediated the relationship between negative emotions and alcohol use and drinking problems in both adolescent and adult samples. Animal studies revealed that increased expression of CRHR1 appears to be crucial for higher stress-induced alcohol intake in genetically selected alcohol-preferring rats (Hansson et al. 2006) as well as in outbred animals in a post-dependent state (Sommer et al. 2008). However, Sillaber et al. (2002) reported that mice lacking functional CRHR1 drank more alcohol, but this phenotype was shown only after repeated exposure to severe stress. Further research has to elucidate the functional role of variation in CRHR1 in humans.

Several limitations in this study are worth noting. First, caution must be exercised in the interpretation of life stress, since it is difficult to separate the effect of environmental factors from genetic liability. Studies using genetically sensitive designs have indicated that many supposed environmental effects actually, in part, reflect genetic factors (Plomin et al. 1994). Thus, exposure to life events may be genetically influenced...
and the G × E interaction observed in the present study might well be due to interactions between the CRHR1 gene and other genes that were not identified. However, this is unlikely in the present study, as there were no significant differences between genotypes regarding stressful life events. Second, while the results appear robust, the sample size is relatively small for a genetic association study. Since association studies are prone to false-positive results, validation in independent samples, as in our previous study (Treutlein et al. 2005; Soyka et al. 2004). This is not surprising, since stress exposure was not considered in these studies, while Sillaber’s and our data suggest that CRHR1 polymorphisms exert their effect on drinking by interacting with stress. In addition, selection of SNPs in the previous studies did not correspond to the haplotypes analysed in our sample, thus limiting comparability of the studies. However, it cannot be ruled out that the observed effect is caused by a population bias, supporting a possible replication in future assessments. In the long run, this problem needs to be overcome by addressing the underlying biological mechanism of the findings. Ultimately, objective measures of physiological, endocrine, and pharmacological responses need to be obtained from the present or other samples, and the functionality of the genetic variants needs to be demonstrated. Third, according to the high-risk design of the study, the participants had been selected for the presence of biological and psychosocial risk factors. Considering that early adversity may moderate the impact of genes on alcohol use, some caution is warranted in generalizing the results to a normal population. Finally, the present findings have to be viewed in the light of the results of previous twin studies which have reported inconsistent, but generally low heritability estimates for alcohol use initiation (Pagan et al. 2006; Rhee et al. 2003), rendering this phenotype somewhat challenging for genetic association studies.

To conclude, the results of this study provide further evidence suggesting that genetic variation in neural pathways mediating the stress response such as the corticotropin-releasing hormone system moderates the impact of life stress on alcohol consumption during adolescence and young adulthood.

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Statement of Interest

None.

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