

ORIGINAL ARTICLE

Genetic association of the human corticotropin releasing hormone receptor 1 (*CRHR1*) with binge drinking and alcohol intake patterns in two independent samples

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To investigate the role of the corticotropin releasing hormone receptor 1 (*CRHR1*) in patterns of human alcohol drinking and its potential contribution to alcohol dependence, we analysed two independent samples: a sample of adolescents, which consisted of individuals from the 'Mannheim Study of Risk Children' (MARC), who had little previous exposure to alcohol, and a sample of alcohol-dependent adults, who met DSM-IV criteria of alcohol dependence. Following determination of allelic frequencies of 14 polymorphisms of the *CRHR1* gene, two haplotype tagging (ht)SNPs discriminating between haplotypes with a frequency of $\geq 0.7\%$ were identified. Both samples were genotyped and systematically examined for association with the htSNPs of *CRHR1*. In the adolescent sample, significant group differences between genotypes were observed in binge drinking, lifetime prevalence of alcohol intake and lifetime prevalence of drunkenness. The sample of adult alcohol-dependent patients showed association of *CRHR1* with high amount of drinking. This is the first time that an association of *CRHR1* with specific patterns of alcohol consumption has been reported. Our findings support results from animal models, suggesting an importance of *CRHR1* in integrating gene-environment effects in alcohol use disorders.

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Introduction

Environmental stress has been suggested to be a risk factor for alcohol abuse, including binge drinking and alcohol dependence.^{1,2} Both clinical and animal studies reported increased drinking following stress.^{3,4} Neuroendocrinological studies provided evidence for a genetic determinant of stress response in alcohol dependence: sons of alcohol-dependent fathers, who have not developed alcohol dependence, show an elevated response to psychosocial stress and are more sensitive to a reduction of the stress response after intake of a moderate dose of alcohol than family negative controls.⁵

The stress reaction is mediated via the hypothalamo-pituitary-adrenocortical (HPA) system. Corticotropin

releasing hormone (CRH) is released from the hypothalamus upon exposure to stressful signals and binds to the corticotropin releasing hormone receptor (CRHR1) in the pituitary gland.⁶ Alcohol intake leads to an increased secretion of CRH and can stimulate HPA axis activity.^{7,8} The activation of the CRHR1 induces the production of second-messenger cAMP in the target cells and stimulates the production of adrenocorticotrophic hormone (ACTH) in the anterior pituitary.⁹ The primary target of ACTH is the adrenal gland, where it binds to the ACTH receptors to release glucocorticoids.

The crucial role of *CRHR1* was impressively demonstrated by a *CRHR1*-knockout model:¹⁰ in the absence of a functional *CRHR1*, the stress response can neither be compensated by any other system, nor by the highly homologous *crhr2* receptor. In addition to the effect of *CRHR1* on HPA-axis activation, a recent study suggests that *CRHR1*-receptors mediate ethanol-induced enhancement of GABAergic synaptic transmission.¹¹

Genetic and environmental influences are hypothesised to have an almost equal contribution to the

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development of alcohol dependence in humans.¹² Given the role of *CRHR1* in mediating the stress response, it may act as a principal integrator of genetic and environmental factors to the development and maintenance of specific patterns of alcohol consumption.

In order to determine the role of *CRHR1* in human alcohol drinking patterns and its possible contribution to alcohol dependence in humans, we investigated two independent samples for association of *CRHR1* and specific alcohol drinking patterns: (a) a sample of adolescents from the 'Mannheim Study of Risk Children' (MARC), who had little previous exposure to alcohol and were assessed for alcohol drinking patterns at the age of 15 years and (b) a sample of alcohol dependent adults, which met DSM-IV criteria of alcohol-dependence. Following determination of allelic frequencies of polymorphisms of the *CRHR1* gene, these two independent samples were genotyped and systematically analysed for association of haplotype tagging single nucleotide polymorphisms (ht)SNPs with patterns of alcohol consumption. Here, we report about the association of *CRHR1* htSNPs with lifetime prevalence of alcohol consumption, lifetime prevalence of drunkenness, frequency of alcohol consumption and lifetime binge drinking in the adolescent sample, and the amount of alcohol intake in the sample of alcohol-dependent adults.

Materials and methods

Subjects and psychiatric assessment

In the first sample (a) 296 participants (153 females, 143 males) of the MARC,¹³ a longitudinal study following children at risk for later psychopathology from birth to adolescence, were investigated. At age 15 years, alcohol consumption during the last 6 months before assessment was measured, using the Lifetime Drinking History Scale (LDH,¹⁴). All participants of the MARC are of Central European descent. Written informed consent was obtained from all participants and their parents. In the second sample 299 patients (mean age 41.58 years; 232 males, 61 females; in six individuals gender was not documented) of central European origin recruited by the Department of Psychiatry of the University of Munich were studied. All patients were treatment seeking, admitted for an inpatient alcohol withdrawal therapy and met DSM-IV criteria for alcohol dependence. Written informed consent was obtained from all individuals when they were in a state of full legal capacity. Interviews were performed by staff members who received intensive rater training. Symptoms related to alcohol dependence were assessed using the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA,¹⁵). The amount of alcohol intake was defined as average alcohol intake 1 week before to admission. It was assessed in 227 patients and was used for stratification (median dichotomisation) into subgroups with high (>250 g/day) or low alcohol

intake (≤ 250 g/day). Both studies were approved by the local ethics committees.

Selection of polymorphisms and in silico analysis of transcription factor binding sites

Selection of the polymorphisms was performed using public SNP databases, based on the criterium of equal distribution along the gene.^{16,17} For those SNPs, that were located in intronic regions of *CRHR1*, hypothetical function was assessed using *in silico* analysis of transcription factor binding sites: both possible alleles of each SNP were tested for their binding capability to human transcription factors.¹⁸ Options employed for the transcription factor binding search using TESS were 21 bases of genomic sequence around each SNP (10 bases on either side of the SNP) and string-based search query with default settings. Recent findings show that transcription factor binding sites may be commonly located in introns or other noncoding regions of the genome.¹⁹ We also ruled out the absence of paralogous intronic regions using electronic polymerase chain reaction (PCR).^{20,21}

Genotype analysis

DNA was prepared from whole blood with standard salting out methods and concentration adjusted with a PicoGreen fluorometric assay (Molecular Probes Inc.). Polymerase chain reaction was performed with HotStarTaq-DNA Polymerase (Qiagen), 4 ng template DNA in a total volume of 25 μ l PCR-reaction. Best oligo pairs were selected for the amplification of each SNP by employing design software on the flanking sequences provided by the SNP databases mentioned above.²² Polymerase chain reaction was performed using standard cycling conditions. Amplified samples were purified using a DNA-purification kit (Invisorb PCR-HTS-96-Kit, Invitek, Berlin) prior to sequencing analysis. Genotyping of the 14 SNPs identified was performed using direct sequencing with an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Genotyping of the htSNPs was performed using RFLP analysis. Of the samples analysed by RFLP, 5–10% were independently replicated by sequencing to ensure the consistency of the genotyping across different analytical methods.

Statistical analysis

Haplotype scoring and htSNP selection, Hardy-Weinberg equilibrium. Haplotypes and frequencies were computed in the 150 individuals using the expectation maximisation (EM) algorithm as implemented in the program COCAPHASE.²³ We then chose a minimal set of two htSNPs which could distinguish all haplotypes with estimated frequency $\geq 0.7\%$. For differences between actual and expected frequencies of the two htSNPs we employed Hardy-Weinberg equilibrium (HWE) equation as implemented in the DeFinetti Program.²⁴

Phenotype variables assessed. Phenotype variables were derived from the instruments mentioned above. Guided by the associations found in the MARC, the amount of alcohol intake was assessed in the sample of the adult alcohol-dependent patients (Table 1).

Univariate and multivariate analyses. Metrical variables were split into groups at the median value. Cochran-Armitage tests were computed in order to examine possible linear trends in the association between the specific single SNPs and the phenotypic variables. Since in most analyses cells with less than five individuals were observed, tests for association between a single SNP and disease status or other drinking behaviour phenotype variables, respectively, were performed using the exact version of the Cochran-Armitage test for trend. In cases where application of a trend test was not possible, a Fisher exact test was carried out. Using a multifactorial analysis (Typ-III analysis), effects of a combination of the two ht-SNPs (4) and (8) were tested in a logistic regression analysis as implemented in SAS 9.1.²⁵

Power analysis. *Post hoc* power analyses was performed under the following assumption: (1) The test used for establishing association was ordinary χ^2 instead of Cochran-Armitage. (2) The proportions compared by means of the χ^2 test were the observed allele frequencies, which is legitimate under HWE.²⁶ The calculations were performed asymptotically using a normal approximation to the exact binomial distribution as implemented in the procedure 'power' that is part of the SAS 9.1 software package.

Results

Genetic analysis of the *CRHR1* gene

Among the genetic variations of the *CRHR1* gene in public databases, we selected 14 SNPs, genotyped them in a group of $n = 150$ healthy individuals. Figure 1 shows the genomic organisation of the *CRHR1* gene and the positions of the genetic variations.

Next, we performed an analysis of linkage disequilibrium (LD)²⁷ (Figure 2) and a frequency analysis (Table 2).

Analysis of the haplotype distribution revealed that two htSNPs were sufficient to discriminate all haplotypes with a frequency $\geq 0.7\%$ (Table 3).

To investigate a possible functional significance of the SNPs identified, a search for transcription factor binding sites using TESS was performed.¹⁸ Six out of the 14 SNPs were found to display a binding difference of transcription factors in an allele-specific manner (Table 4).^{28–34}

The six SNPs with potential function are all grouped in one haplotype block, which is defined by htSNP (8), as shaded grey in Table 3. htSNP (8) integrates all potentially functional genetic variations investigated in this study.

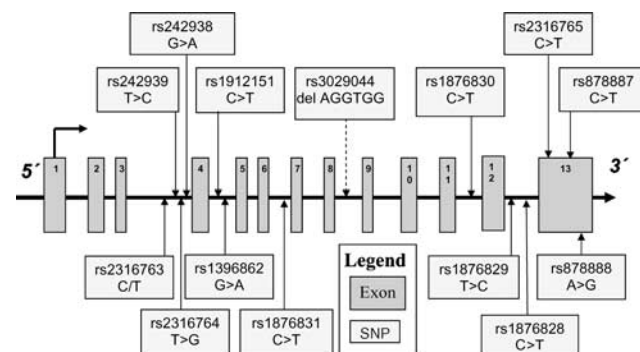


Figure 1 Schematic representation of the exon-intron structure of the *CRHR1* gene. Genomic organisation of the gene with 13 exons (depicted as grey coloured rectangles and identified with Arabic numerals) and 12 introns (indicated by horizontal lines connecting the boxes, not drawn to scale), localisation of the polymorphisms, nomenclature of the SNPs (continuous arrows) and insertion/deletion polymorphisms (Insdel; dotted arrow) according to dbSNP database (Reference SNP IDs-numbers) and alleles.

Table 1 Phenotypic variables and description in Mannheim Study of Risk Children adolescent and adult-alcohol dependent samples

Variable	Description
<i>Adolescent sample</i>	
Lifetime prevalence of alcohol drinking	Subject has tried alcohol never or only once throughout the life vs more often
Lifetime prevalence of being drunk	Subject has never been drunk vs has been drunk
Frequency of alcohol consumption	Up to once per month vs more than once per month
Lifetime binge drinking	Binge drinkers are subjects who had ≥ 5 (female ≥ 4) drinks per occasion at least once in their lifetime
<i>Munich patients sample</i>	
Alcohol intake	Average amount of alcohol consumed per day in grams in the last 7 days before admission

Association of htSNPs with alcohol consumption and binge drinking in the adolescent sample

The consumption of the first complete glass of alcohol of the adolescent subjects of the MARC study was at an average age of 13.23 ± 1.05 years. The subjects were assessed for an association of the tagging SNPs (4) and (8) with various alcohol consumption patterns (Table 5a). Confirmatory analyses were performed for lifetime binge drinking, whereas lifetime prevalence of alcohol consumption and drunkenness as well as frequency of alcohol consumption were analysed in an exploratory way. SNP (4) was associated with binge drinking and with lifetime prevalence of drunkenness (Table 5a). Significant group differences in genotypes of SNP (8) were observed in binge

drinking and in lifetime prevalence of alcohol intake as well as lifetime prevalence of drunkenness (Table 5a). For the clinically relevant parameter of binge drinking, the risk genotype found for htSNP (4) was AA, for htSNP (8) CC. No association of SNPs (4) and (8) with frequency of alcohol consumption (Table 5a) and with measures assessing age of onset of drinking behaviour (data not shown) was observed.

Taken together, our results of the association analysis in the adolescent sample suggest a relevance of *CRHR1* genotypes for the amount of drinking (binge drinking), but not for the frequency of alcohol drinking.

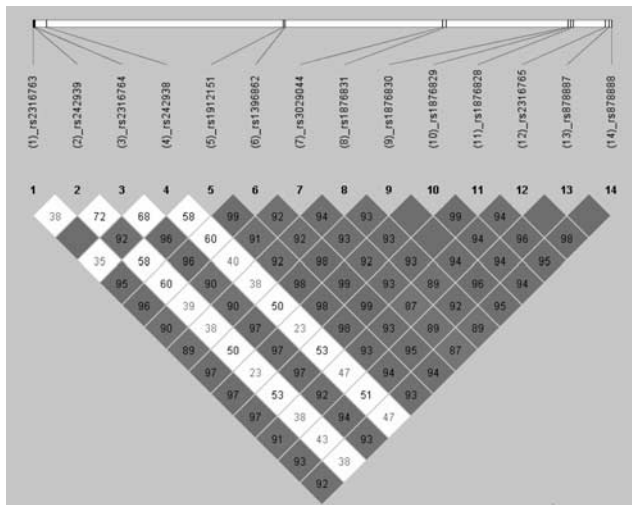


Figure 2 Linkage disequilibrium (LD) structure,⁵⁷ LD colour scheme of the *CRHR1* gene (red: high D' , $LOD \geq 2$; white: low D' , $LOD < 2$) and localisation of the 14 SNPs analysed. D' values of 1.0 are represented by the empty squares.

Table 3 Haplotype estimates and frequencies of haplotypes

Haplotype SNP	1	2	3
(1) rs2316763	1	2	1
(2) rs242939	1	1	2
(3) rs2316764	1	2	1
(4) rs242938	1	1	2
(5) rs1912151	1	2	1
(6) rs1396862	1	2	1
(7) rs3029044	1	2	1
(8) rs1876831	1	2	1
(9) rs1876830	1	2	1
(10) rs1876829	1	2	1
(11) rs1876828	1	2	1
(12) rs2316765	1	2	1
(13) rs878887	1	2	1
(14) rs878888	1	2	1
Frequency	0.670	0.150	0.066

Haplotype tagging (ht) single nucleotide polymorphisms in bold. (1) indicates the allele with major frequency, (2) the allele with minor frequency. The different shades indicate the haplotype blocks represented by SNP 4 and SNP 8, respectively.

Table 2 Nucleotide exchanges, positions, and allele frequencies of genetic variations of the *CRHR1* gene

SNP/Insdel ID	Nucleotide variation	Position on Chr 17 (NCBI mapviewer)	Localization	Minor allele frequency
(1) rs2316763	C to T	44370950	Intron	0.20
(2) rs242939	T to C	44370999	Intron	0.09
(3) rs2316764	T to G	44371022	Intron	0.18
(4) rs242938	G to A	44371356	Intron	0.10
(5) rs1912151	C to T	44378364	Intron	0.19
(6) rs1396862	G to A	44378417	Intron	0.19
(7) rs3029044	del/AGGTGG	44383062	Intron	0.19
(8) rs1876831	C to T	44383165	Intron	0.18
(9) rs1876830	C to T	44386772	Intron	0.18
(10) rs1876829	T to C	44386863	Intron	0.20
(11) rs1876828	C to T	44386945	Intron	0.20
(12) rs2316765	T to C	44387874	UTR	0.18
(13) rs878887	C to T	44388002	UTR	0.19
(14) rs878888	A to G	44388055	UTR	0.19

Table 4 Analysis of alteration of binding motives for transcription factors by single nucleotide polymorphisms^{28–34}

SNP	Allele 1 (corresponding to htSNP (8) C allele)	Allele 2 (corresponding to htSNP (8) T allele)	Function
(1) rs2316763	—	—	
(2) rs242939	—	—	
(3) rs2316764	—	—	
(4) rs242938	—	—	
(5) rs1912151	c-Myb (C allele)	(T allele)	Neuronal survival
(6) rs1396862	(G allele)	TCF-1alpha (A allele)	Transcriptional activation
(7) rs3029044	(Deletion allele)	c-Myc (Insertion allele)	Growth regulation/apoptosis
(8) rs1876831	Sp1 (G allele)	(A allele)	Transcriptional activation
(9) rs1876830	COUP (C allele)	Sp1 (T allele)	Transcriptional inhibition (COUP), Transcriptional activation (SP1)
(10) rs1876829	(T allele)	GCF (C allele)	Transcriptional inhibition
(11) rs1876828	—	—	
(12) rs2316765	—	—	
(13) rs878887	—	—	
(14) rs878888	—	—	

Abbreviation: htSNP, haplotype tagging single nucleotide polymorphisms.

The different shades indicate the haplotype blocks represented by SNP 4 and SNP 8, respectively.

In order to detect a possible interaction of both htSNPs, a logistic regression analysis was performed. Although this multivariate analysis confirmed the associations detected in the univariate analyses, no evidence for an interaction of the two genotypes could be observed: with the genotypes collapsed (frequent homozygotes against a group of heterozygotes and rare homozygotes), logistic regression analysis (split into two groups at the median) revealed that only htSNP(8), but not htSNP(4) contributes to binge drinking (htSNP(4): $P=0.1365$; htSNP(8): $P=0.0108$; $P(\text{global beta})=0.0043$).

Association of htSNPs with enhanced alcohol intake in the sample of adult alcohol-dependent patients

Next we assessed the role of *CRHR1* htSNPs in an independent sample of adult alcohol dependent patients. We hypothesised that the risk genotypes of *CRHR1* htSNPs (4) and (8) are also associated with the increased alcohol intake in adult alcohol-dependent patients. In our second sample, patients with high alcohol intake (>250 g/day) were compared to those drinking less than or equal to 250 g/day. The results support our hypothesis in the case of htSNP (8) (Armitage trend test $P=0.0444$), but did not reach statistical significance in the case of htSNP (4) (Table 5b).

In order to assess the risk of a possible false positive result, we performed *post hoc* power analyses of SNP 8, which contributes most to the association observed in our two samples. Whereas the adolescent sample had 91% power to detect effects of the observed OR of 2.242 with a $P<0.05$, the sample of adult alcohol-dependent patients had 39% power to detect the observed OR of 1.506 with a $P<0.05$. Both samples had >90% power to detect the OR of the upper confidence limit (Table 6).

Discussion

In the present study, a systematic analysis of 14 genetic variations of the CRH-receptor 1 gene was performed and two htSNPs were identified, which discriminate between all estimated haplotypes with a frequency of $f\geq 0,7\%$. These htSNPs were used to genotype an adolescent sample and to analyse associations with patterns of alcohol consumption. In a confirmatory analysis, we found an association with patterns of binge drinking, which was mainly driven by htSNP (8). Our sample had 91% power to detect $\alpha<0.05$ at the OR observed. Exploratory analyses revealed associations with lifetime prevalence for drunkenness for both SNPs as well as lifetime prevalence for alcohol intake in SNP (8). No association was found with measures of frequency of drinking. In a second independent analysis, preliminary evidence is provided for an association of SNP (8) with the amount of alcohol intake in a sample of adult alcohol-dependent patients.

Of particular clinical relevance is the association of both htSNPs with binge drinking, which is an increasingly popular form of alcohol abuse.³⁵ In various European countries, binge drinking among adolescents has a prevalence rate between 24 and 32%.³⁶ Owing to its acute and chronic effects, binge drinking raises to be a major public health issue, representing an especially malign form of alcohol abuse.

In addition to the problems arising from acute alcohol intoxications and related diseases,³⁷ binge drinking is associated with a particularly bad prognosis in terms of later alcohol misuse and alcohol dependence: In a study of 2387 individuals, binge drinking during adolescence was associated with binge drinking at ages 30 or 31 years for both men

Table 5 (a) Phenotypic variables of alcohol drinking patterns, number of cases genotyped, genotype distributions, and association analysis (genotype-specific for *p*-values, allele-specific for OR values) of drinking patterns in Mannheim Study of Risk Children children with htSNPs (4) and (8). (b) Amount of alcohol intake, number of cases genotyped, distribution of genotypes, and association analysis (genotype-specific for *p*-values, allele-specific for OR values) of the amount of drinking (high vs low, median split at 250 g/day) in alcohol-dependent patients with htSNPs (4) and (8)

Phenotype	Cases genotyped	Distribution of genotypes			P-value	Odds ratio (95% CI)
		GG	GA	AA		
(a)						
<i>htSNP (4) rs242938</i>						
Lifetime binge drinking (male ≥ 5 drinks; female ≥ 4 drinks)	Yes: 93	71 76.34%	20 21.51%	2 2.15%	0.0134 (Armitage, two-sided)	2.127 (1.179–3.838)
	No: 192	168 87.50%	23 11.98%	1 0.52%		
Lifetime prevalence, drunkenness	Yes: 116	89 76.72%	25 21.55%	2 1.72%	0.0074 (Armitage, two-sided)	2.271 (1.251–4.123)
	No: 169	150 88.76%	18 10.65%	1 0.59%		
Frequency of alcohol consumption	>1/week: 38	31 81.58%	6 15.79%	1 2.63%	0.4254 (Fisher's exact test)	NA
	≤ 1 /week: 70	56 80.00%	13 18.57%	1 1.43%		
	≤ 1 /month:177	152 85.88%	24 13.56%	1 0.56%		
Lifetime prevalence, alcohol	Yes: 213	177 83.10%	33 15.49%	3 1.41%	0.5032 (Armitage, two-sided)	1.350 (0.656–2.780)
	No: 72	62 86.11%	10 13.89%	0 0.0%		
(b)						
<i>htSNP (8) s1876831</i>						
Lifetime binge drinking (male ≥ 5 drinks; female ≥ 4 drinks)	Yes: 91	70 76.92%	20 21.98%	1 1.10%	0.0018 (Armitage, two-sided)	2.242 (1.353–3.718)
	No: 191	113 59.16%	66 34.55%	12 6.28%		
Lifetime prevalence, drunkenness	Yes: 114	85 74.56%	26 22.81%	3 2.63%	0.0059 (Armitage, two-sided)	1.916 (1.220–3.003)
	No: 168	98 58.33%	60 35.71%	10 5.95%		
Frequency of alcohol consumption	>1/week: 37	28 75.68%	8 21.62%	1 2.70%	0.3738 (Fisher's exact test)	NA
	≤ 1 /week: 68	48 70.59%	17 25.00%	3 4.41%		
	≤ 1 /month:177	107 60.45%	61 34.46%	9 5.08%		
Lifetime prevalence, alcohol	Yes: 211	145 68.72%	59 27.96%	7 3.32%	0.0123 (Armitage, two-sided)	1.812 (1.159–2.833)
	No: 71	38 53.52%	27 38.03%	6 8.45%		
(b)						
<i>htSNP (4) rs 242938</i>						
Low intake	117	90 76.92%	27 23.08%	0 0.0%	0.0993 (Armitage, one-sided)	NA
High intake	110	93 84.55%	17 15.45%	0 0.0%		

Table 5 *continued*

Phenotype	Cases genotyped	Distribution of genotypes			P-value	Odds ratio (95% CI)
		CC	CT	TT		
<i>htSNP (8) rs 1876831</i>						
Low intake	116	66 56.90%	49 42.24%	1 0.86%	0.0444 (Armitage, one-sided)	1.506 (0.935–2.421)
	111	78	31	2		
High intake		70.27%	27.93%	1.80%		

Abbreviation: htSNP, haplotype tagging single nucleotide polymorphisms; HWE, Hardy–Weinberg equilibrium.

HWE (exact) for htSNP (4) was $p=0.446184$ and for htSNP (8) $p=0.457329$.

Hardy–Weinberg disequilibrium (exact test) was determined for all patients in the study, even if the phenotypic variable alcohol intake was not present, with the genotype distributions: htSNP (4): GG: 232; GA: 61; AA: 0; htSNP (8): CC: 184; CT: 104; TT: 6. HWE calculated was for htSNP (4) $p=0.054677$ and for htSNP (8) $p=0.063304$. OR (alcohol intake) for htSNP (8) is 0.558, with CI = (0.323–0.966).

Table 6 *Post hoc* power analysis of the observed effects for haplotype tagging single nucleotide polymorphisms (8)

	Power ^a
<i>Binge drinking in adolescents</i>	
Observed odds ratio	0.914
Lower confidence interval	0.263
Upper confidence interval	0.999
<i>Alcohol intake in adult patients</i>	
Observed odds ratio	0.393
Lower confidence interval	0.060
Upper confidence interval	0.919

^aPower to detect effects with $\alpha < 0.05$.

and women, generating relative risks of 2.3 and 3.0, respectively.³⁸ Bonomo *et al.*³⁹ reported, that higher persisting teenage rates of binge drinking preceded alcohol dependence in young adults. Another recent study confirmed the predictive value of binge drinking: binge drinking patterns, which were exhibited during the college years posed significant risk factors for alcohol dependence and abuse 10 years after the initial interview.⁴⁰

A strong correlation of binge drinking with environmental factors has been described: frequent binge drinking is more closely associated with mental distress, including stress, depression, and emotional problems than other forms of alcohol use.⁴¹ Another study suggests that there is a strong association between use of alcohol to cope with tension and binge drinking.⁴²

Taken together, our work provides evidence for a genetic contribution of a *CRHR1* genotype to binge drinking and suggests an important role of *CRHR1* in integrating gene–environment effects in humans. However, to confirm this hypothesis, additional studies need to be conducted, which analyse samples specifically phenotyped for gene–environment interactions.

In humans, *CRH* is known to mediate unconditioned and conditioned anxiogenic-like behavioural responses to stressor exposure and, thus, influence drug reinforcement and dependence.^{43,44} Exposure to stressors as well as maladaptive responses to stress increase alcohol drinking and relapse behaviour in humans.^{45–47} In congruence with these observations a recent study reported that mice lacking a functional *CRHR1* showed enhanced and delayed stress-induced alcohol drinking, which persisted for at least 6 months.⁴ The penetrance of the signal resulting from the genetic constitution of *CRHR1* in adolescents up to alcohol dependence in the adults, which was found in our samples, supports the persistent effect observed on alcohol drinking in the study by Sillaber *et al.*⁴

On the basis of the results of our study, hypotheses as to the molecular mechanisms resulting in a differential activity of *CRHR1* can be generated. For example, *CRHR1* SNP 8 alters an intronic binding site for transcription factor Sp1, which regulates transcriptional activation.^{48,49} Intronic transcription factor binding sites, which contribute to intronic enhancers or intronic silencers were found in several genes,^{50,51} including the human serotonin transporter gene,^{52,53} and could also contribute to transcriptional regulation of *CRHR1*. Therefore, alteration of the Sp1 binding site by SNP 8 may lead to a genotype–specific transcriptional activation resulting in differential amounts of available *CRHR1* receptors. Interestingly, a point mutation or deletion of a consensus Sp1 binding site greatly reduces the transcriptional activation of an ethanol responsive gene *hsc70*.⁵⁴ Both, the transcription factor Sp1⁵⁵ as well as *CRHR1*⁵⁶ are implicated in plasticity and behaviour and may play a role in the long-term behavioural adaptation to ethanol.⁵⁷ On the basis of these findings as well as the behavioural studies in humans and *CRHR1* knockout mice, a mechanistic explanation of our results can be proposed: altered availability of *CRHR1* receptor caused by the allelic state of SNP 8 could predispose juveniles to alcohol drinking upon stress-

ful stimuli, and exhibit a long lasting effect leading to manifest alcohol dependence classified with DSM-IV in adulthood.

In two other recent studies, no association was found between *CRHR1* polymorphisms and alcohol dependence.^{58,59} The selection of SNPs in the study of Soyka *et al.*,⁵⁸ which analysed personality traits derived from the Cloninger type 1 definition of alcohol-dependent patients, including harm avoidance, reward dependence and novelty seeking, does not correspond to the 14 SNPs represented by our htSNPs, which limits the comparability with our results. Dahl *et al.*⁵⁹ analysed five SNPs of *CRHR1*, of which two, namely rs1396862 and rs878887, correspond to our SNPs (6) and (13), respectively. In contrast to our study, Dahl *et al.*⁵⁹ tested a relatively small population of 120 alcohol-dependent individuals, which were characterised only for their clinical diagnosis, without further dissection of the specific patterns of alcohol consumption. Therefore, and in particular because alcohol dependence is not a homogeneous disorder,⁶⁰ the discrepancies of the results point towards the use of defined phenotype patterns for association analyses and the necessity for development of exact phenotype definitions, based on biological criteria.

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References

- Aseltine Jr RH, Gore SL. The variable effects of stress on alcohol use from adolescence to early adulthood. *Subst Use Misuse* 2000; **35**: 643–668.
- Schmidt LG, Dufeu P, Kuhn S, Smolka M, Rommelspacher H. Transition to alcohol dependence: clinical and neurobiological considerations. *Compr Psychiatry* 2000; **41**: 90–94.
- de Wit H, Soderpalm AH, Nikolayev L, Young E. Effects of acute social stress on alcohol consumption in healthy subjects. *Alcohol Clin Exp Res* 2003; **27**: 1270–1277.
- Sillaber I, Rammes G, Zimmermann S, Mahal B, Zieglerberger W, Wurst W *et al.* Enhanced and delayed stress-induced alcohol drinking in mice lacking functional CRH1 receptors. *Science* 2002; **296**: 931–933.
- Zimmermann U, Spring K, Kunz-Ebrecht SR, Uhr M, Wittchen HU, Holsboer F. Effect of ethanol on hypothalamic-pituitary-adrenal

- system response to psychosocial stress in sons of alcohol-dependent fathers. *Neuropsychopharmacology* 2004; **29**: 1156–1165.
- Bittencourt JC, Sawchenko PE. Do centrally administered neuropeptides access cognate receptors: an analysis in the central corticotropin-releasing factor system. *J Neurosci* 2000; **20**: 1142–1156.
- de Waele JP, Gianoulakis C. Effects of single and repeated exposures to ethanol on hypothalamic beta-endorphin and CRH release by the C57BL/6 and DBA/2 strains of mice. *Neuroendocrinology* 1993; **57**: 700–709.
- Haddad JJ. Alcoholism and neuro-immune-endocrine interactions: physicochemical aspects. *Biochem Biophys Res Commun* 2004; **323**: 361–371.
- Borrelli E. A chilled-out knockout. *Nat Genet* 1998; **19**: 108–109.
- Timpl P, Spanagel R, Sillaber I, Kresse A, Reul JM, Stalla GK *et al.* Impaired stress response and reduced anxiety in mice lacking a functional corticotropin-releasing hormone receptor 1. *Nat Genet* 1998; **19**: 162–166.
- Nie Z, Schweitzer P, Roberts AJ, Madamba SG, Moore SD, Siggins GR. Ethanol augments GABAergic transmission in the central amygdala via CRF1 Receptors. *Science* 2004; **303**: 1512–1514.
- Enoch MA, Goldman D. Genetics of alcoholism and substance abuse. *Psychiatr Clin North Am* 1999; **22**: 289–299.
- Laucht M, Esser G, Baving L, Gerhold M, Hoesch I, Ihle W *et al.* Behavioral sequelae of perinatal insults and early family adversity at 8 years of age. *J Am Acad Child Adolesc Psychiatry* 2000; **39**: 1229–1237.
- Skinner HA, Scheu WJ. Reliability of alcohol use indices: the lifetime drinking and the MAST. *J Stud Alcohol* 1982; **43**: 1157–1170.
- Buchholz KK, Cadoret R, Cloninger CR, Dinwiddie SH, Hesselbrock VM, Nurnberger Jr JI *et al.* A new, semi-structured psychiatric interview for use in genetic linkage studies: a report on the reliability of the SSAGA. *J Stud Alcohol* 1994; **55**: 149–158.
- Sherry ST, Ward MH, Kholodov M, Baker J, Phan L, Smigielski EM *et al.* dbSNP: the NCBI database of genetic variation. *Nucleic Acids Res* 2001; **29**: 308–311.
- Riva A, Kohane IS. SNPper retrieval and analysis of human SNPs. *Bioinformatics* 2002; **18**: 1681–1685.
- Schug J, Overton GC. TESS: Transcription element search software on the WWW. Technical report CBIL-TR-1997-1001-v0.0 Computational Biology and Informatics Laboratory School of Medicine: University of Pennsylvania, PA, USA, 1997.
- Long F, Liu H, Hahn C, Sumazin P, Zhang MQ, Zilberstein A. Genome-wide prediction and analysis of function-specific transcription factor binding sites. *In Silico Biol* 2004; **4**: 395–410.
- Gut IG, Lathrop GM. Duplicating SNPs. *Nat Genet* 2004; **36**: 789–790.
- Schuler GD. Sequence mapping by electronic PCR. *Genome Res* 1997; **7**: 541–550.
- Rozen S, Skaletsky HJ. Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S (eds). *Bioinformatics Methods and Protocols: Methods in Molecular Biology*. Humana Press: Totowa, NJ, 2000, pp 365–386.
- Dudbridge F. Pedigree disequilibrium tests for multilocus haplotypes. *Genet Epidemiol* 2003; **25**: 115–221.
- Strom Wienker. DeFinetti program online at <http://ihg.gsf.de/cgi-bin/hw/hwa1.pl>, 2005.
- SAS Institute Inc. *SAS 9.1.3 Help and Documentation*. SAS Institute Inc.: Cary, NC, 2000–2004.
- Sasieni PD. From genotypes to genes: doubling the sample size. *Biometrics* 1997; **53**: 1253–1261.
- Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005; **21**: 263–265.
- Vorbrueggen G, Kalkbrenner F, Guehmann S, Moelling K. The carboxyterminus of human c-myc protein stimulates activated transcription in trans. *Nucleic Acids Res* 1994; **22**: 2466–2475.
- Carlsson P, Waterman ML, Jones KA. The hLEF/TCF-1alpha HMG protein contains a context-dependent transcriptional activation domain that induces the TCRalpha enhancer in T cells. *Genes Dev* 1993; **7**: 2418–2430.
- Oosterwegel M, van de Wetering M, Timmerman J, Kruisbeek A, Destree O, Meijlink F, *et al.* Differential expression of the HMG box

- factors TCF-1 and LEF-1 during murine embryogenesis. *Development* 1993; **118**: 439–448.
- 31 Tani E, Kitagawa H, Ikemoto H, Matsumoto T. Proteasome inhibitors induce Fas-mediated apoptosis by c-Myc accumulation and subsequent induction of FasL message in human glioma cells. *FEBS Lett* 2001; **504**: 53–58.
- 32 Schwartz C, Catez P, Rohr O, Lecestre D, Aunis D, Schaeffer E. Functional interactions between C/EBP, Sp1, and COUP-TF regulate human immunodeficiency virus type 1 gene transcription in human brain cells. *J Virol* 2000; **74**: 65–73.
- 33 Tran P, Zhang X-K, Salbert G, Hermann T, Lehmann JM, Pfahl M. COUP orphan receptors are negative regulators of retinoic acid response pathways. *Mol Cell Biol* 1992; **12**: 4666–4676.
- 34 Kageyama R, Pastan I. Molecular cloning and characterization of a human DNA binding factor that represses transcription. *Cell* 1989; **59**: 815–825.
- 35 Pincock S. Binge drinking on rise in UK and elsewhere. Government report shows increases in alcohol consumption, cirrhosis, and premature deaths. *Lancet* 2003; **362**: 1126–1127.
- 36 European School Survey Project on Alcohol and Other Drugs (ESPAD). Collected data on young people's alcohol habits; last wave in 2003 included 35 countries. Summary of the 2003 findings.
- 37 Gowda RM, Khan IA, Vasavada BC, Sacchi TJ. Alcohol-triggered acute myocardial infarction. *Am J Ther* 2003; **10**: 71–72.
- 38 McCarty CA, Ebel BE, Garrison MM, DiGiuseppe DL, Christakis DA, Rivara FP. Continuity of binge and harmful drinking from late adolescence to early adulthood. *Pediatrics* 2004; **114**: 714–719.
- 39 Bonomo YA, Bowes G, Coffey C, Carlin JB, Patton GC. Teenage drinking and the onset of alcohol dependence: a cohort study over seven years. *Addiction* 2004; **99**: 1489–1490.
- 40 Jennison KM. The short-term effects and unintended long-term consequences of binge drinking in college: a 10-year follow-up study. *Am J Drug Alcohol Abuse* 2004; **30**: 659–684.
- 41 Okoro CA, Brewer RD, Naimi TS, Moriarty DG, Giles WH, Mokdad AH. Binge drinking and health-related quality of life: do popular perceptions match reality? *Am J Prev Med* 2004; **26**: 230–233.
- 42 Tyssen R, Vaglum P, Aasland OG, Gronvold NT, Ekeberg O. Use of alcohol to cope with tension, and its relation to gender, years in medical school and hazardous drinking: a study of two nationwide Norwegian samples of medical students. *Addiction* 1998; **93**: 1341–1349.
- 43 Steckler T, Holsboer F. Corticotropin-releasing hormone receptor subtypes and emotion. *Biol Psychiatry* 1999; **46**: 1480–1508.
- 44 Sarnyai Z, Shaham Y, Heinrichs SC. The role of corticotropin-releasing factor in drug addiction. *Pharmacol Rev* 2001; **53**: 209–243.
- 45 Brown SA, Inaba RK, Gillin JC, Schuckit MA, Stewart MA, Irwin MR. Alcoholism and affective disorder: clinical course of depressive symptoms. *Am J Psychiatry* 1995; **152**: 45–52.
- 46 Kreek MJ, Koob GF. Drug dependence: stress and dysregulation of brain reward pathways. *Drug Alcohol Depend* 1998; **51**: 23–47.
- 47 Junghanns K, Backhaus J, Tietz U, Lange W, Bernzen J, Wetterling T et al. Impaired serum cortisol stress response is a predictor of early relapse. *Alcohol Alcohol* 2003; **38**: 189–193.
- 48 Morgan WD. Transcriptional factor Sp1 binds to and activates a human hsp70 gene promoter. *Mol Cell Biol* 1989; **9**: 4099–4104.
- 49 Anderson GM, Freytag SO. Synergistic activation of a human promoter *in vivo* by transcription factor Sp1. *Mol Cell Biol* 1991; **11**: 1935–1943.
- 50 Suhasini M, Reddy CD, Reddy EP, DiDonato JA, Pilz RB. cAMP-induced NF-kappaB (p50/relB) binding to a c-myc intronic enhancer correlates with c-myc up-regulation and inhibition of erythroleukemia cell differentiation. *Oncogene* 1997; **15**: 1859–1870.
- 51 Sato A, Keng VW, Yamamoto T, Kasamatsu S, Ban T, Tanaka H et al. Identification and characterization of the hematopoietic cell-specific enhancer-like element of the mouse hex gene. *J Biochem (Tokyo)* 2004; **135**: 259–268.
- 52 Fiskerstrand CE, Lovejoy EA, Quinn JP. An intronic polymorphic domain often associated with susceptibility to affective disorders has allele dependent differential enhancer activity in embryonic stem cells. *FEBS Lett* 1999; **458**: 171–174.
- 53 MacKenzie A, Quinn J. A serotonin transporter gene intron 2 polymorphic region, correlated with affective disorders, has allele-dependent differential enhancer-like properties in the mouse embryo. *Proc Natl Acad Sci USA* 1999; **96**: 15251–15255.
- 54 Wilke N, Sganga MW, Gayer GG, Hsieh KP, Miles MF. Characterization of promoter elements mediating ethanol regulation of hsc70 gene transcription. *J Pharmacol Exp Ther* 2000; **292**: 173–180.
- 55 Mittal N, Nathan JB, Pandey SC. Neuroadaptational changes in DNA binding of stimulatory protein-1 and nuclear factor-kB gene transcription factors during ethanol dependence. *Eur J Pharmacol* 1999; **386**: 113–119.
- 56 Rainnie DG, Bergeron R, Sajdyk TJ, Patil M, Gehlert DR, Shekhar A. Corticotropin releasing factor-induced synaptic plasticity in the amygdala translates stress into emotional disorders. *J Neurosci* 2004; **24**: 3471–3479.
- 57 Rulten SL, Ripley TL, Hunt CL, Stephens DN, Mayne LV. Sp1 and NFkappaB pathways are regulated in brain in response to acute and chronic ethanol. *Genes, Brain and Behavior* 2005, (in press).
- 58 Soyka M, Preuss UW, Koller G, Zill P, Hesselbrock V, Bondy B. No association of CRH1 receptor polymorphism haplotypes, harm avoidance and other personality dimensions in alcohol dependence: results from the Munich gene bank project for alcoholism. *Addict Biol* 2004; **9**: 73–79.
- 59 Dahl JP, Doyle GA, Oslin DW, Buono RJ, Ferraro TN, Lohoff FW et al. Lack of association between single nucleotide polymorphisms in the corticotropin releasing hormone receptor 1 (*CRHR1*) gene and alcohol dependence. *J Psychiatr Res* 2005; **39**: 475–479.
- 60 Davidson KM, Ritson EB. The relationship between alcohol dependence and depression. *Alcohol Alcohol* 1993; **28**: 147–155.