ONLINE FIRST

Prediction of the Risk of Comorbid Alcoholism in Schizophrenia by Interaction of Common Genetic Variants in the Corticotropin-Releasing Factor System

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Context: Stress plays a major role in the development of comorbid alcohol use disorder (AUD). In turn, AUD worsens the outcome of psychiatric patients with respect to global disease severity, social situation, and socioeconomic burden. Prediction of persons at risk for AUD is crucial for future preventive and therapeutic strategies.

Objective: To investigate whether genetic variants of the corticotropin-releasing factor system or their interaction influence the risk of developing AUD in chronic disease populations.

Design: Genotype analysis comprising selected single-nucleotide polymorphisms within the CRHR1 and CRHBP genes in patients with schizophrenia and in a nonschizophrenic psychiatric disease control sample should allow the extraction of predictors of comorbid AUD. Gene expression (messenger RNA) analysis in peripheral blood mononuclear cells was performed to gain the first mechanistic insight.

Setting: An ideal setup for this study was the Göttingen Research Association for Schizophrenia Data Collection of schizophrenic patients, specifically intended to enable association of genetic information with quantifiable phenotypes in a phenotype-based genetic association study.

Patients: A total of 1037 schizophrenic patients (Göttingen Research Association for Schizophrenia sample), 80 nonschizophrenic psychiatric disease controls as a small replicate sample, and a case-control study including 1141 healthy subjects.

Main Outcome Measures: Association of CRHR1 and CRHBP genotypes with the following: (1) AUD; (2) a newly developed alcoholism severity score comprising 5 AUD-relevant variables; and (3) quantitative CRHR1 and CRHBP messenger RNA expression.

Results: An interaction of CRHR1 rs110402 and CRHBP rs3811939 predicts high risk of comorbid AUD in schizophrenic patients (odds ratio=2.27; 95% confidence interval, 1.56-3.30; \( P = .001 \)) as well as psychiatric disease controls (odds ratio=4.02; 95% confidence interval, 0.95-17.05; \( P = .06 \)) and leads to the highest CRHR1/CRHBP messenger RNA ratio (\( P = .02 \); dysbalanced stress axis).

Conclusions: The high predictive value of a genetic interaction within the stress axis for the risk of comorbid AUD may be used for novel preventive and individualized therapeutic approaches.

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Alcohol Use Disorders (AUDs) are severe, complex illnesses with prevalence rates of up to 30%.1-3 Treatment of AUD is hampered by high relapse rates after clinical detoxification and months of abstinence.4-6 In psychiatric diseases like schizophrenia, comorbid AUDs reach an even more dramatic prevalence. In the Epidemiologic Catchment Area Study, 47% of schizophrenic patients fulfilled criteria of any substance use disorder.3 Even though numbers become smaller once AUD alone is considered (34%), individuals with schizophrenia are at high risk for developing AUD.7-10 Comorbid AUD in turn deteriorates the course of disease and outcome, eg, it causes a higher percentage of housing problems,11 disability,12 and hospital admissions.13

Any severe disease poses a tremendous stress on the affected individual. The high amount of comorbid substance abuse in schizophrenia may be the result of a dysfunctional way of coping with this stress. The use of alcohol as an easily available tool to reduce tension and handle nega-
tive emotions in the sense of self-treatment plays an essential role in AUD etiology.14-18 Both inborn and acquired capacities to respond to stress are likely to influence this process. Of central importance for a coordinated stress response in mammals is the hypothalamus-pituitary-adrenal axis, with the corticotropin-releasing factor system playing a dominant role. Components of this highly interregulated system include corticotropin-releasing hormone (CRH),19 CRH receptors,20 and CRH-binding protein.21 The latter represents a passive ligand trap that neutralizes CRH by binding it, thereby terminating its biological actions, in contrast to its active receptor that initiates signal transduction on binding.22

While long-term alcohol consumption can induce lasting alterations within the CRH system,23,24 less is known about how genetic variation of respective genes influences development of AUD. A pivotal study in alcohol-naive mice demonstrated that Crhr1 null mutation was associated with augmented ethanol consumption on stress exposure.25 Conversely, in ethanol-dependent mice, reduction of Crhr1 activity by Crhr1 blockade or by Crhr1 null mutation led to decreased alcohol self-administration.26 In rhesus macaques, higher alcohol intake was found in animals carrying a CRH genotype conferring increased stress reactivity.27

In humans as well, the CRH system has been linked not only to depression,28,29 suicidality,30 and panic disorder31 but also to alcohol consumption and AUD.32-36 Specifically, associations were described for a North American Caucasian population between AUD and 3 single-nucleotide polymorphisms (SNPs) in the CRHR1 gene.32 Similarly, the Mannheim Study of Children at Risk observed relationships between CRHR1 SNPs and alcohol consumption patterns, eg, binge drinking and lifetime prevalence of drunkenness.33 Based on DSM-IV37 diagnosis only, a recent study found an association of genetic variants of the CRHR1 gene with AUD.34 Also, protective constellations of CRHR1 regarding stress-related AUD exist.35,36

This study was designed to investigate associations of genetic variants within the CRH system including their interaction with the development of AUD in a chronic disease population. Schizophrenia, a severe psychiatric disorder as a grave and persistent stessor shared by the cohort under study, should allow for defining predictors of comorbid AUD. To test this hypothesis, the Goettingen Research Association for Schizophrenia (GRAS) Data Collection was used, specifically intended to enable association of genetic information with quantifiable phenotypes in a procedure termed phenotype-based genetic association study (PGAS).38,39 Based on GRAS/PGAS, we report here for the first time to our knowledge an interactive genetic constellation within the CRH system comprising variants of CRHR1 and CRHBP with high predictive value to detect an increased risk of comorbid AUD in schizophrenic patients. Importantly, we simultaneously provide replication of this finding in a psychiatric disease control cohort purposely comprising patients with different psychiatric diagnoses excluding schizophrenia. This heterogeneous replicate sample underscores the generalizability of the revealed risk constellation for imperiled populations.

METHODS

STUDY SETTING AND PARTICIPANTS

Schizophrenic Patients

Study participants were enrolled in the cross-sectional field study of GRAS as described previously.38,39 The study was approved by the Ethics Committee of the Georg-August-University, Goettingen, Germany, and review boards of participating centers, and it complies with the Declaration of Helsinki. The GRAS Data Collection comprises at present 1037 patients with confirmed DSM-IV diagnosis of schizophrenia (82.2%) or schizoaffective disorder (17.8%) examined between September 1, 2005, and November 1, 2010, in 23 collaborating centers across Germany (Table 1).30,39 Almost all of these patients were of European Caucasian descent (93.6% Caucasian, 1.6% other ethnicities, and 2.8% unknown). European Caucasian persons are a genetically homogeneous group with low average levels of genetic differentiation compared with other human populations (no strong influence on association results to be expected).39,42 Specifically, the German population is very homogeneous, with low genetic differentiation along a north-south gradient within Germany. In fact, population substructure within Germany is too low to be detectable without prior information on subpopulation membership.43 Therefore, for the purpose of our study, population stratification was not essential.

Psychiatric Disease Controls

As an independent, nonschizophrenic disease control (replicate) sample, 80 patients with mental disorders other than schizophrenia (57.5% affective disorder, 16.3% substance use disorder [including multiple drug or cannabis use], 10.0% anxiety disorder, 6.3% personality disorder, 3.7% delusional disorder, 3.7% organic mental disorders, and 2.5% mental retardation—all diagnosed according to DSM-IV) were recruited in Gottingen (eTable 1, http://www.archgenpsychiatry.com).

Healthy Controls

Healthy controls exclusively for the genetic case-control part of the study were voluntary blood donors (n=1141) recruited according to national guidelines for blood donation. As such, they widely fulfill health criteria, ensured by broad predonation screening containing standardized questionnaires, interviews, and determinations of hemoglobin level, blood pressure, pulse, and body temperature. Comparable to the patient population, almost all control subjects were of European Caucasian descent (97.8% Caucasian, 2.0% other ethnicities, and 0.2% unknown).38,39

PHENOTYPING

Comprehensive interviews, testing, and clinical ratings were conducted by one and the same traveling team of trained examiners (psychiatrists, psychologists) using the GRAS Manual.38,39 Additionally, records and discharge letters of every patient were used to validate and complement the patient’s (and, if applicable, relative’s or caretaker’s) statements.

Sociodemographic and Clinical Variables

Semi-structured interviews delivered biographic data, family background, level of education, and occupational history. Diagnoses of schizophrenia or schizoaffective disorders were based on the
Table 1. Göttingen Research Association for Schizophrenia Sample Description

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total GRAS Sample (n=1037)</th>
<th>No AUD (n=673)</th>
<th>AUD (n=364)</th>
<th>P Value (Z/χ² Value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sociodemographic</td>
<td></td>
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</tr>
<tr>
<td>Age, y</td>
<td>39.52 (12.56) [17.49-79.49]</td>
<td>39.20 (12.03) [17.49-79.49]</td>
<td>39.62 (12.86) [17.49-79.49]</td>
<td>.01 (Z=−2.45)</td>
</tr>
<tr>
<td>Male, %</td>
<td>693 (66.8)</td>
<td>399 (59.3)</td>
<td>294 (80.8)</td>
<td>.001 (χ²=49.18)</td>
</tr>
<tr>
<td>Caucasian, %</td>
<td>990 (95.6)</td>
<td>648 (96.3)</td>
<td>342 (94.0)</td>
<td>.18 (χ²=4.85)</td>
</tr>
<tr>
<td>Education, y</td>
<td>12.01 (3.05) [8-27]</td>
<td>12.25 (3.18) [8-27]</td>
<td>11.57 (2.73) [8-23]</td>
<td>.001 (Z=−3.19)</td>
</tr>
<tr>
<td>Unemployed, %</td>
<td>368 (38.3)</td>
<td>214 (34.0)</td>
<td>154 (46.2)</td>
<td>&lt;.001 (χ²=35.81)</td>
</tr>
<tr>
<td>Inpatient at assessment, %</td>
<td>445 (42.9)</td>
<td>262 (38.9)</td>
<td>183 (50.3)</td>
<td>&lt;.001 (χ²=21.03)</td>
</tr>
<tr>
<td>Clinical</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at first episode, y</td>
<td>26.25 (8.79) [5.40-67.55]</td>
<td>26.74 (8.16) [7.96-67.55]</td>
<td>25.33 (8.01) [5.40-75.79]</td>
<td>.02 (Z=−2.25)</td>
</tr>
<tr>
<td>Duration of disease (first episode), y</td>
<td>13.24 (10.70) [0.01-58.44]</td>
<td>13.56 (11.22) [0.04-58.44]</td>
<td>12.64 (9.68) [0.01-49.28]</td>
<td>.61 (Z=−0.51)</td>
</tr>
<tr>
<td>Age at onset (prodrome), y</td>
<td>23.28 (8.69) [2.02-62.31]</td>
<td>23.84 (9.01) [2.02-62.31]</td>
<td>22.15 (7.92) [6.78-55.44]</td>
<td>.005 (Z=−2.82)</td>
</tr>
<tr>
<td>Duration of disease (prodrome), y</td>
<td>15.97 (11.12) [0.05-62.31]</td>
<td>16.17 (11.55) [0.05-62.31]</td>
<td>15.57 (10.26) [0.06-52.28]</td>
<td>.82 (Z=−0.23)</td>
</tr>
<tr>
<td>Chlorpromazine equivalents</td>
<td>690.11 (711.17) [0-7350.00]</td>
<td>688.99 (729.49) [0-7375.00]</td>
<td>692.17 (656.88) [0-6324.29]</td>
<td>.28 (Z=−1.07)</td>
</tr>
<tr>
<td>Diagnosis of schizophrenia, No. (%)</td>
<td>852 (82.2)</td>
<td>584 (81.4)</td>
<td>304 (83.5)</td>
<td>.40 (χ²=0.70)</td>
</tr>
<tr>
<td>Hospitalizations, No.</td>
<td>8.60 (9.77) [0-97]</td>
<td>7.76 (9.20) [0-82]</td>
<td>10.16 (10.57) [0-97]</td>
<td>&lt;.001 (Z=−4.88)</td>
</tr>
<tr>
<td>PANSS score</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>13.76 (6.32) [7-38]</td>
<td>13.67 (6.47) [7-38]</td>
<td>13.93 (6.94) [7-36]</td>
<td>.18 (Z=−1.35)</td>
</tr>
<tr>
<td>Negative</td>
<td>18.23 (7.83) [7-46]</td>
<td>18.21 (7.85) [7-44]</td>
<td>18.35 (7.87) [7-46]</td>
<td>.91 (Z=−0.11)</td>
</tr>
<tr>
<td>General</td>
<td>33.73 (11.83) [16-82]</td>
<td>33.61 (11.91) [16-78]</td>
<td>33.96 (11.70) [16-82]</td>
<td>.54 (Z=−0.61)</td>
</tr>
<tr>
<td>Total</td>
<td>65.64 (23.40) [30-160]</td>
<td>65.33 (23.58) [30-148]</td>
<td>65.87 (23.58) [30-160]</td>
<td>.48 (Z=−0.49)</td>
</tr>
<tr>
<td>GAF score</td>
<td>45.76 (17.25) [5-90]</td>
<td>46.90 (17.89) [5-90]</td>
<td>43.67 (15.82) [9-90]</td>
<td>.01 (Z=−2.44)</td>
</tr>
<tr>
<td>CGI score</td>
<td>5.57 (1.08) [2-8]</td>
<td>5.52 (1.09) [2-8]</td>
<td>5.66 (1.06) [2-8]</td>
<td>.03 (Z=−2.12)</td>
</tr>
<tr>
<td>Addiction-specific</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of cigarettes/d</td>
<td>16.90 (14.97) [0-80]</td>
<td>13.47 (14.18) [0-80]</td>
<td>23.17 (14.34) [0-80]</td>
<td>&lt;.001 (χ²=10.16)</td>
</tr>
<tr>
<td>No. (%)</td>
<td>717 (70.5)</td>
<td>399 (60.7)</td>
<td>318 (88.3)</td>
<td>&lt;.001 (χ²=85.20)</td>
</tr>
<tr>
<td>Multiple drug use according to DSM-IV, No. (%)</td>
<td>101 (9.7)</td>
<td>24 (3.8)</td>
<td>77 (21.2)</td>
<td>&lt;.001 (χ²=83.12)</td>
</tr>
<tr>
<td>Cannabis use disorder according to DSM-IV, No. (%)</td>
<td>338 (32.6)</td>
<td>143 (21.2)</td>
<td>195 (53.6)</td>
<td>&lt;.001 (χ²=112.34)</td>
</tr>
<tr>
<td>Benzodiazepine use disorder</td>
<td>39 (3.8)</td>
<td>17 (2.5)</td>
<td>22 (6.0)</td>
<td>.004 (χ²=8.08)</td>
</tr>
<tr>
<td>Alcohol in lifetime, g/d</td>
<td>57.79 (103.51) [0-1224.50]</td>
<td>5.41 (9.13) [0-70.56]</td>
<td>124.25 (129.12) [3.86-1224.50]</td>
<td>&lt;.001 (Z=−22.62)</td>
</tr>
<tr>
<td>Alcohol-related detoxifications, No.</td>
<td>0.19 (0.22) [0-20]</td>
<td>0</td>
<td>0.55 (1.94) [0-20]</td>
<td>&lt;.001 (Z=−10.55)</td>
</tr>
<tr>
<td>Chronicity, problematic drinking, y/age, y</td>
<td>0.14 (0.22) [0-0.79]</td>
<td>0.01 (0.06) [0-0.59]</td>
<td>0.41 (0.18) [0.03-0.79]</td>
<td>&lt;.001 (Z=−28.45)</td>
</tr>
<tr>
<td>Daily drinking, No. (%)</td>
<td>291 (32.7)</td>
<td>28 (5.0)</td>
<td>263 (79.5)</td>
<td>&lt;.001 (Z=−22.82)</td>
</tr>
<tr>
<td>SCID yes answers</td>
<td>3.12 (4.08) [0-14]</td>
<td>3.01 (0.72) [0-10]</td>
<td>3.08 (4.27) [0-14]</td>
<td>&lt;.001 (Z=−19.67)</td>
</tr>
</tbody>
</table>

Abbreviations: AUD, alcohol use disorder; CGI, Clinical Global Impression; GAF, Global Assessment of Functioning; GRAS, Göttingen Research Association for Schizophrenia; PANSS, Positive and Negative Symptom Scale; SCID, Structured Clinical Interview for DSM-IV Disorders.

Due to missing data on phenotyping, sample sizes vary between 747 and 1037 in the total sample, between 424 and 673 in patients without AUDs, and between 296 and 364 in patients with AUDs.

For statistical methods, Mann-Whitney U or χ² tests were used. Bolded values, P < .05.

Exploratory exclusion of non-Caucasian subjects did not appreciably alter any of the main findings of the study.

Rating according to graduation/certificate; patients currently in school or in educational training are excluded.

Versus schizoaffective disorders.

Components of the alcoholism severity score.

Addiction section of the SCID (criteria for abuse and dependence).

Structured Clinical Interview for DSM-IV Disorders and substantiated by information from medical records, which also conveyed numbers and durations of hospital stays and age at onset of schizophrenia and prodrome. Psychopathological state, symptom severity, and functional outcome were evaluated by clinical ratings (Positive and Negative Syndrome Scale, Clinical Global Impression scale, and Global Assessment of Functioning) and questionnaires (State-Trait Anxiety Inventory and Brief Symptom Inventory). For appraising current depression, 2 items of the Positive and Negative Syndrome Scale general psychopathology subscale (guilt feelings and depression) were used, together with 2 items of the Brief Symptom Inventory (guilt feelings and thoughts of death or dying) and the Brief Symptom Inventory depression subscale. For judging the degree of anxiety, the Positive and Negative Syndrome Scale general items (anxiety, tension, and somatic concern), Brief Symptom Inventory (anxiety scale), and State-Trait Anxiety Inventory were used (figure 1).

Alcohol-Related Variables

Two main outcome measures were used: (a) the dichotomous DSM-IV AUD diagnosis summarizing alcohol abuse and dependence; and (b) a newly developed quantitative alcohol severity score. For assessing AUD, the Structured Clinical Interview for DSM-IV Disorders (addiction section) was applied. Patient statements were confirmed and supplemented with longitudinal information (records and discharge letters). In the alcoholism severity score, 3 alcohol-relevant variables were integrated (≥3 of 5 variables were required for calculating the score): (1) numbers of alcohol-related detoxifications; (2) highest amount of regular drinking (in grams per day for ≥6 months); (3) frequency of drinking (11-point scale from never to daily); (4) number of positive Structured Clinical Interview for DSM-IV Disorders items; and (5) chronicity (years of problematic alcohol use divided by age in years).
GENOTYPING

Single-nucleotide polymorphisms in CRHR1 and CRHBP were selected, 3 of each gene, considering database information on minor allele frequencies (NCBI, HapMap, UCSC Genome Browser) and previous reports, which had identified these SNPs to be informative (Figure 1A). Genotyping was performed with Simple Probes (TIB Molbiol, Berlin, Germany) on a Light Cycler 480 (Roche, Mannheim, Germany) (Appendix). Successful genotyping of the GRAS sample (n=1037) ranged from n=1016 to n=1030 (average 98%), accounting for some variation of respective n numbers.

EXPRESSION ANALYSIS IN PERIPHERAL BLOOD MONONUCLEAR CELLS

Isolation of Peripheral Blood Mononuclear Cells

Blood of a number of schizophrenic GRAS patients and psychiatric disease controls (n=104; 64.4% male; 51.0% schizophrenic) was collected in tubes with a citrate phosphate dextrose adenine solution. Peripheral blood mononuclear cells were isolated with the Ficoll-Paque PLUS isolation procedure (GE Healthcare, Munchen, Germany).

Quantitative Reverse Transcription–Polymerase Chain Reaction

We prepared RNA with the miRNeasy Mini Kit (Qiagen, Hilden, Germany) and used it to synthesize complementary DNA (SuperScriptIII; Invitrogen, Karlsruhe, Germany). Quantitative reverse transcription–polymerase chain reaction was performed with SYBR Green detection on the LightCycler 480 system (Roche). Cycle threshold values were standardized to ß-actin (Appendix).

STATISTICAL ANALYSES

Group differences in categorical and continuous variables were assessed with nonparametric Mann-Whitney U and χ² tests, respectively. Blom transformation was used to yield standardized values being approximately normally distributed with a mean of 0 and variance of 1. Intercorrelations and internal consistency of alcoholism severity score components were assessed using Pearson correlation coefficient and Cronbach α. To assess the association between alcoholism severity score and AUD (continuous and binary variable), the point-biserial correlation was calculated. Analysis of covariance (adjusted for age) was used to analyze the effect of SNPs on the standardized alcoholism severity score.
and its single items. For the dichotomous outcome AUD, estimation of the odds ratio (OR) and its 95% confidence interval (CI) for the effect of SNPs was performed in a logistic regression model incorporating age as an additional confounder. For all analyses, statistical significance was set to the .05 level. Statistical analyses were performed using SPSS for Windows version 17.0 statistical software (SPSS Inc, Chicago, Illinois) and R version 2.10.1 statistical software (R Foundation, Vienna, Austria).

RESULTS

AUD DIAGNOSIS IN THE GRAS POPULATION OF SCHIZOPHRENIC PATIENTS

To explore a potential role of the 3 CRHR1 and 3 CRHBP SNPs as genetic risk factors for schizophrenia, a case-control study was conducted. No significant difference in distribution of genotypes between cases (GRAS patients, n=1037) and healthy controls (n=1141) was found for any of the 6 SNPs (eTable 2).

OPERATIONALIZATION OF THE ALCOHOLISM SEVERITY SCORE IN THE GRAS SAMPLE

For more detailed genotype-phenotype associations, the refined alcoholism severity score on top of the dichotomous AUD diagnosis was created. Intercorrelations between selected target variables are presented in Figure 1B. A high internal consistency of these variables (Cronbach α=.878) justifies their handling as an alcoholism severity score. However, whereas diagnosis of AUD was available for all 1037 GRAS patients, the alcoholism severity score could be determined for only 957 patients (for 80 patients, <3 of 5 variables were obtainable). Correlation between AUD and the alcoholism severity score amounts to r=0.85 (Figure 1C).

CASE-CONTROL STUDY

To explore a potential role of the 3 CRHR1 and 3 CRHBP SNPs as genetic risk factors for schizophrenia, a case-control study was conducted. No significant difference in distribution of genotypes between cases (GRAS patients, n=1037) and healthy controls (n=1141) was found for any of the 6 SNPs (eTable 2).

PHENOTYPE-BASED GENETIC ASSOCIATION STUDY

GRAS Patients

The hypothesis-guided PGAS approach started with association analyses of alcohol-relevant readouts with selected SNPs (Table 2). Only CRHR1 SNP1 (rs110402) turned out to be associated with the alcoholism severity score (F_{2,926}=5.60; P=.004), whereas all other markers showed no associations or only tendencies, eg, SNP1 of CRHBP (rs3811939) (F_{1,925}=2.32; P=.10). When considering associations of SNPs with individual target variables, however, more hits arise: all 3 SNPs of CRHR1 and SNP1 of CRHBP are associated with consumed alcohol in grams per day. Genotype-related distributions of raw data are displayed in eFigure 2. No associations between the 6 SNPs and disease-related or disease-unrelated control variables were detected. Neither anxiety nor depression score yielded significant results. Except for numbers of cigarettes (F_{2,926}=3.93; P=.02), no associations between any of the SNPs and other drugs were uncovered. Importantly, after correction for alcoholism severity score (as a covariate), the significant relationship between smoking and CRHR1 SNP1 disappeared (F_{1,926}=2.18; P=.11).

We next checked, based on the known biological interplay of CRHR1 and CRHBP, a potential interaction of the most prominently alcoholism severity score-associated SNP1 genotypes of each gene. Figure 2A illustrates the genotype-phenotype results for CRHR1 SNP1 (rs110402) and CRHBP SNP1 (rs3811939) separately: the TT carriers and the GG carriers, respectively, have the highest association with the alcoholism severity score. On grouping for interaction, a high-risk genotype for comorbid AUD as judged by the alcoholism severity score, consisting of homozygous T in CRHR1 SNP1 (rs110402) and homozygous G in CRHBP SNP1 (rs3811939), contrast a significantly lower risk of all other possible combinations (F_{1,312}=15.13, P<.001; F_{1,201}=8.64, P=.004; F_{1,58}=11.81, P=.001). The interaction between CRHR1 SNP1 and CRHBP SNP1 in the analysis of covariance model was strong (F_{1,255}=6.34; P=0.1) (Figure 2B). Also, on the level of AUD diagnosis, this interaction is obvious. Risk genotype carriers have a higher proportion of AUD (OR=2.27; 95% CI, 1.56-3.30; P<.001) (Figure 2C). For comparison, CRHR1 SNP1 alone as risk factor yields an OR of 1.62 (95% CI, 1.20-2.20; P=.002), and CRHBP SNP1 has an OR of 0.89 (95% CI, 0.69-1.16; P=.40).

Nonschizophrenic Psychiatric Disease Controls/Replicate Sample

To explore whether a comparable risk vs nonrisk constellation would be detectable in a nonschizophrenic population, we analyzed a small psychiatric disease control sample (n=80). This sample differs from the GRAS population (n=1037) expectedly in several ways. Whereas the percentage of AUD is relatively comparable (40.0% vs 35.1% in the GRAS sample), disease controls have lower rates of unemployment, lower doses of antipsychotics, lower Positive and Negative Syndrome Scale subscale

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scores, and better functional outcome (according to the Global Assessment of Functioning) and smoke fewer cigarettes per day (eTable 1). Despite the small number and somewhat different characteristics, a pattern very similar to the GRAS sample becomes obvious in disease controls with respect to both alcoholism severity score and AUD diagnosis (Figure 2D). Again, risk genotype carriers tend to have a substantially higher alcoholism severity score compared with all other genotype combinations ($F_{1,41} = 4.88$, $P = .03$; $F_{1,17} = 4.59$, $P = .05$; $F_{1,1} = 3.80$, $P = .06$). Owing to the small sample size, the interaction effect of CRHR1 SNP1 and CRHBP SNP1 on alcoholism severity score just failed to reach statistical significance ($F_{1,180} = 2.48; P = .12$). Also regarding AUD diagnosis, risk genotype carriers tend to differ from noncarriers (OR=4.02, 95% CI, 0.95-17.05; $P = .06$) (Figure 2C).

**GENOTYPE-DEPENDENT EXPRESSION ANALYSIS IN PERIPHERAL BLOOD MONONUCLEAR CELLS**

To gain the first mechanistic insight, peripheral blood mononuclear cells were isolated from a number of schizophrenia patients and psychiatric disease controls (total of n=104) available for blood sampling, and CRHR1 and CRHBP messenger RNA (mRNA) levels were quantified. As a biological estimate of ligand efficiency or activity of the CRH system, the mRNA expression ratio of CRHR1 (active receptor) and CRHBP (ligand trap) was used.

CRHR1 and CRHBP compete for binding of CRH. Whereas the former is the active receptor that mediates the effects of CRH, the latter acts as a ligand trap, catching CRH and thereby preventing the bound molecule from having a biological effect at its receptor. In other words, the ratio of active receptor and binding protein is of high importance for the quantitative biological effect of a major determinant of the stress axis. Indeed, patients carrying the risk genotype combination (TT/GG) have a significantly higher ratio of CRHR1 to CRHBP mRNA ($Z = -2.31; P = .02$), ie, a putative dysbalance of the CRH system (Figure 2E). Separating this subgroup into subjects with or without AUD, an almost identical pattern of genotype-dependent mRNA expression was obtained, even though it failed to reach statistical significance owing to the small sample numbers (Figure 2E).
These data underscore a genotype-related rather than purely alcohol-induced mRNA expression difference.

**COMMENT**

We identified a prominent interaction of distinct variants of 2 genes of the CRH system, CRHR1 and CRHBP, predicting the risk of comorbid AUD in a chronically stressed population. In more than 1000 schizophrenic subjects, we showed that carriers of a homozygous T allele in CRHR1 rs110402 combined with a homozygous G allele in CRHBP rs3811939 are more than twice as likely to develop comorbid AUD (OR = 2.27) than carriers of all other possible genotype combinations (which may even be seen as protective regarding AUD). Moreover, we already replicated this finding in a smaller nonschizophrenic psychiatric disease control group, emphasizing the general importance of this observation for populations under chronic stress. In the case-control study, comparing frequencies of genotype distribution in healthy and schizophrenic individuals, none of these genotypes plays a role as schizophrenia risk factor.

An experimental approach to chronic stress–induced alcoholism in humans is very difficult to take. In this study, schizophrenia was used as a model of severe chronic stress in a field-study-type design. Patients

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Figure 2. Phenotype-based genetic association study. A, Distribution of alcoholism severity scores in CRHR1 SNP1 genotypes and CRHBP SNP1 genotypes using analysis of covariance adjusted for age. GRAS indicates Göttingen Research Association for Schizophrenia. Data are presented as mean (SEM). B, Interaction effect between CRHR1 SNP1 and CRHBP SNP1 genotypes with respect to alcoholism severity score in the GRAS sample using analysis of covariance adjusted for age. Data are presented as mean (SEM). C, Interaction effect between CRHR1 SNP1 and CRHBP SNP1 genotypes with respect to the diagnosis of alcohol use disorder (AUD) according to DSM-IV using logistic regression analyses with age as confounder for estimating odds ratios (ORs) and 95% confidence intervals (CIs). D, Interaction effect between CRHR1 SNP1 and CRHBP SNP1 genotypes with respect to alcoholism severity score in psychiatric disease controls using analysis of covariance adjusted for age. Data are presented as mean (SEM). E, Ratio of CRHR1 and CRHBP messenger RNA (mRNA) expression in peripheral blood mononuclear cells (PBMCs) dependent on genotypes (risk genotype against all others) in a total of 104 patients as well as on separation of these patients according to the diagnosis of AUD (n = 34) and non-AUD (n = 70) using Mann-Whitney U tests. Raw data of mRNA levels (normalized to β-actin) dependent on genotype combinations are presented in the table below.
with schizophrenia (as heterogeneous as this disease may be) and its social and personal consequences certainly belong to an endangered population with respect to their chronic stress level. Nevertheless, the fact that stress might be a causative factor of alcoholism in schizophrenia (and other chronic diseases) does not exclude further mechanisms leading to increased alcohol consumption in this and other disease populations. Also, we have to be aware that stress is a very complex biological system and the corticotropin system is only part of it. Therefore, the interaction detected here between CRHR1 and CRHBP will not explain each and every aspect of stress-induced alcoholism; however, it will provide clinically and prophylactically important information for those who carry the risk constellation of genotypes.

Except for SNP1 of CRHR1, single markers of CRHR1 and CRHBP show no or only the tendency of an association with AUD or the alcoholism severity score. The most prominent effect is obtained by combining genotypes CRHR1 rs110402 and CRHBP rs3811939. Importantly, gene expression levels in peripheral blood mononuclear cells reveal quantitative differences that may explain biological consequences of respective genotype combinations: the ratio of CRHR1 to CRHBP mRNA is highest in the risk constellation, independent of the presence or absence of AUD. This finding supports the primary (genetic) influence on basal gene expression in the sense of an innate dysbalanced (hyperactive) stress axis, which may be additionally challenged by long-term alcohol consumption.

We note that the detected associations are restricted to AUD and do not extend to other drug use disorders, eg, cannabis use, benzodiazepine use, or polytoxicomania. An apparent association of CRHR1 rs110402 with cigarette smoking disappears after correction for AUD, underscoring the high comorbidity of smoking and AUD. Nevertheless, because nicotine is a stimulator of the stress axis and alcohol and nicotine dependence have an overlapping genetic background, this association may deserve further elucidation.

The finding of a high predictive power of 2 genotypes within the CRH system not only substantiates the biological role of stress in AUD development but may also serve as an indicator of persons at risk. Comorbid AUD adds to the negative outcome of schizophrenia and socioeconomic burden. Thus, intensified preventive measures for high-risk subjects and perhaps even personalized genotype-based treatment strategies might be desirable.

The presence of comorbid AUD in as many as 35% of the GRAS population and the devastating psychosocial and clinical consequences agree well with other reports on schizophrenic patients. The causal role of AUD in this overall aggravatetion situation is emphasized by the fact that schizophrenia-typical symptoms are not different between AUD and non-AUD groups. Surprisingly, at first view, patients with AUD have an earlier age at onset of schizophrenic prodrome and first schizophrenic episode compared with patients without AUD. This finding, however, may be explained by more cannabis use disorders in patients with AUD because cannabis but not alcohol is clearly associated with earlier age at onset. Accordingly, correcting for cannabis use eliminates the association of AUD with age at onset in the GRAS population.

For decades, it has been known that AUD has a heritability of 50% to 60%. Regarding genetic risk factors, the genes of the CRH system and their interaction belong to a whole group of genes that predict the risk of developing alcoholism. For instance, genome-wide association studies on AUD including up to 2000 patients have identified genes encoding alcohol dehydrogenase, γ-aminobutyric acid A receptor, dopamine receptor, and serotonin receptor to be associated (OR < 2) with the risk of alcoholism. However, as with other complex psychiatric diseases, the diagnosis alone (as used in genome-wide association studies) is of limited value in spotting relevant genetic risk constellations and even less helpful for identifying important biological subgroups of the disorder.

Interplay between AUD and stress has been demonstrated in several animal experiments. First human studies indicated that CRH receptor antagonists may reduce symptom severity of depression and anxiety and improve resistance against psychosocial stress. Treatment of AUD with CRH receptor ligands is presently under study in clinical trials (eg, ClinicalTrials.gov identifier NCT01187511). Such an approach should be specifically tested in the herein delineated subgroup of at-risk individuals. Contrasting reports on other disease populations, no associations of the herein described risk genotype with depression and anxiety were found in schizophrenic patients. This negative finding may be related to the underlying disease phenotype, the instruments used, or the fact that respective symptoms were determined cross-sectionally.

To conclude, our data suggest that a distinct genotype constellation comprising 2 determinants of the CRH system has high power to predict the risk of comorbid AUD in endangered populations. This knowledge should be used for preventive strategies in patients with severe psychiatric disease to avert further individual health, social, or economic decline. Moreover, it could deliver a basis for novel individualized treatment approaches with, for example, CRH antagonists.
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REFERENCES


