

Involvement of the atrial natriuretic peptide transcription factor *GATA4* in alcohol dependence, relapse risk and treatment response to acamprosate

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Received 14 January 2010; revised 30 March 2010; accepted 27 April 2010

In alcoholism, both relapse to alcohol drinking and treatment response are suggested to be genetically modulated. This study set out to determine whether the top 15 single nucleotide polymorphisms (SNPs) of a recent genome-wide association (GWA) and follow-up study of alcohol dependence are associated with relapse behavior and pharmacological treatment response in 374 alcohol-dependent subjects who underwent a randomized, double-blind, placebo-controlled trial with acamprosate, naltrexone or placebo. The single nucleotide polymorphism, rs13273672, an intronic SNP in the gene for GATA-binding protein 4 (*GATA4*), was associated with relapse within the 90-day medical treatment period ($P < 0.01$). Subsequent pharmacogenetic analyses showed that this association was mainly based on patients treated with acamprosate ($P < 0.01$). In line with the observation that natriuretic peptide promoters are modulated by *GATA4*, a significant gene dose effect on the variance of atrial natriuretic peptide (ANP) plasma concentration in the different *GATA4* genotypes ($P < 0.01$) was found. Hence, genetic variations in *GATA4* might influence relapse and treatment response to acamprosate in alcohol-dependent patients via modulation of ANP plasma levels. These results could help to identify those alcohol-dependent patients who may be at an increased risk of relapse and who may better respond to treatment with acamprosate.

The Pharmacogenomics Journal advance online publication, 29 June 2010; doi:10.1038/tpj.2010.51

Keywords: genetic association study; alcohol; *GATA4*; atrial natriuretic peptide; acamprosate

Introduction

A large body of genetic epidemiological data strongly implicates genetic factors in the etiology of alcohol dependence. One promising approach to identify such underlying susceptibility genes is the systematic genome wide association study (GWAS), which uses a high number (500 000+) of single nucleotide polymorphisms (SNPs) across the genome, and which has now been successfully conducted in a variety of complex disorders (for all published GWA studies, see: www.genome.gov/26525384). We recently conducted a GWA and a follow-up study of alcohol dependence.¹ To arrive at our selection of SNPs for the follow-up study, we used a 'top down approach' including the 121 SNPs with the smallest P -values, and—using a convergent animal approach—further included 19 SNPs, which showed less significant associations but were located in the human homologs of rat genes that had previously shown differential expression in the

rat brain after chronic and excessive alcohol consumption. A total of 15 SNPs showed associations with the same alleles as in the GWAS.¹ Nine of these SNPs were located in different genes and three of these intragenically located SNPs had been included due to the animal approach. The finding that three out of 22 SNPs from the animal approach were replicated in the follow-up study is very unlikely to be due to chance ($P = 1.69 \times 10^{-2}$) and demonstrates the potency of this strategy. Of the SNPs identified by this approach, one was located in the *alcohol dehydrogenase (ADH)* gene cluster, which belongs to the most consistently replicated loci contributing to alcohol-related phenotypes.² The second one was located in *Cadherin13 (CDH13)*, which has been described in previous studies as a susceptibility locus for alcohol dependence³ and which is hypothesized to constitute a common susceptibility gene for addiction.⁴ The third SNP was located in the *GATA binding protein 4 (GATA4)* gene. The *GATA4* gene represents a transcription factor regulating the transcription of the atrial natriuretic peptide (ANP),⁵ a peptide repeatedly shown to be involved in the pathophysiology of alcohol dependence.^{6,7}

As alcohol addiction results from being unable to refrain from repetitive drinking despite considerable efforts to do so, it seems plausible that genetic variants that are involved in the etiology of alcohol dependence may also influence the ability to refrain from drinking during therapeutic intervention.

One conclusion drawn, however, from human genetic studies of addiction is that genes relevant to the development of alcohol or drug dependence may not necessarily also influence the probability of successful cessation once a level of heavy drinking or drug taking has been reached.⁸ This conclusion is in accordance with the general hypothesis that genes involved in the acquisition of drug taking behavior (reinforcement) only partially overlap with genes that impact on relapse.⁹ In this study, we therefore investigated whether the 15 most promising susceptibility variants shown to be involved in alcohol dependence are also involved in modulating the risk of relapse in patients participating in a randomized, placebo-controlled, relapse prevention trial with acamprosate and naltrexone.¹⁰ Aiming to predict treatment responses under either acamprosate or naltrexone, we also examined possible pharmacogenetic interactions with the SNP found to be associated with relapse in the present study.

Materials and methods

Participants

All 374 patients included in this investigation were participants of the PREDICT study for whom genotype information was available. The PREDICT study is a randomized, double-blind, placebo-controlled multicenter trial conducted in Germany,¹⁰ which closely resembled the COMBINE study¹¹ regarding methodology, assessment and intervention. The sub-sample of this study did not differ significantly from the PREDICT population regarding sex

ratio ($\chi^2(1) < 0.001$, $P > 0.05$), age at study enrollment ($t(793) = -0.55$, $P > 0.05$; M (present sample) = 44.92, s.d. = 8.55) and age of dependence onset ($t(771) = 0.06$, $P > 0.05$). The patients' average age at enrollment was 45 years. All patients fulfilled a DSM-IV (Diagnostic and Statistical Manual, Fourth Edition)/ICD 10 (International Classification of Diseases) diagnosis of alcohol dependence. After a manualized inpatient alcohol-withdrawal program, the participants were randomly assigned to a double-blind treatment with acamprosate (1998 mg per day), naltrexone (50 mg per day) or placebo using an imbalanced randomization algorithm ensuring proportions of 2:2:1 for acamprosate, naltrexone and placebo, respectively. Medication was given for 3 months in addition to supportive counseling (medical management) aimed at enhancing compliance. Daily alcohol use and abstinence status was assessed using a standardized self-report questionnaire (Form 90), measures of blood alcohol levels, liver enzymes (aspartate amino transferase, alanine amino transferase, gamma-glutamyltransferase) and carbohydrate-deficient transferrin and independent corroboration. All participants provided written informed consent according to the Principles of Declaration of Helsinki. The study was approved by the ethics committee of the University of Heidelberg. Further details of the study design have been published elsewhere.¹⁰

Outcome criteria

The outcome criteria were occurrence of a relapse to heavy drinking and time to relapse to heavy drinking within the 90-day treatment period. In line with the main outcome criteria of the PREDICT study, heavy drinking was defined as consumption of more than 48 g per day alcohol for females and 60 g per day alcohol for males. This *a priori* selected outcome criterion was chosen because it represents one of the phenotypes most often investigated in treatment efficacy studies. No other phenotypes were analyzed in this study.

DNA preparation and genotyping

In the original GWA analysis, 15 of 140 variants carried forward to the follow-up study showed associations with the same alleles as observed in the GWA study (for details of the study see Treutlein *et al.*¹). In this study, we tested whether these 15 candidate SNPs show an association with relapse after detoxification in the PREDICT-study participants. Genomic DNA was prepared from whole blood according to standard procedures. Genotyping was performed by primer extension reaction chemistry with matrix-assisted laser desorption/ionization-time of flight mass spectrometry using the iPLEX Assay (Sequenom, San Diego, CA, USA) at the Life and Brain Center, University of Bonn. Microtiter plates contained a mixture of patients and controls; two individuals (2.1%) were genotyped as technical controls on each plate, and genotype replicate consistency was 100%. A standardized quality control procedure was applied to the raw data, that is, the genotype scatterplot (used as a graphical representation of the normalized probe intensities of each marker) was visually assessed by two investigators.

Plasma preparation and ANP analyses

The patients' blood samples, which were collected between 7 and 14 days after detoxification and before the randomization to and initiation of pharmacological treatment with acamprosate, naltrexone or placebo, were cooled and anticoagulated with EDTA. After centrifugation, plasma was separated and stored at -80°C until analysis. Atrial natriuretic peptide level was measured after extraction with octadecasilica-silyl cartridges (Supelco, Bellefonte, PA, USA) using a radioimmuno assay kit (Phoenix Pharmaceuticals, Burlingame, CA, USA) in 42 trios (AA, AG and GG) matched according to age, sex, and age of onset. The detection limit was 8 pg per ml plasma; intra- and interassay coefficients of variation were below 10% at 80 pg per ml.

Statistical data analysis

Data analysis was performed using the SAS/STAT software version 9.2 (SAS Institute, Cary, NC, USA), R version 2.7.2 (<http://www.R-project.org>) and SPSS release 15.0 (SPSS, Chicago, IL, USA). Single SNP association tests (two-tailed, significance level set at 0.05) were carried out using the Cochran–Armitage test for linear trend in proportions. Tests for deviation from Hardy–Weinberg equilibrium were performed using an exact test.¹² Differences in survival distribution functions were assessed using the logrank test. To test for homogeneity of variance of ANP plasma concentrations between genotypes, a modified Levene test based on trimmed means was applied.

Analysis of linkage disequilibrium (LD) structure and search for functional signatures of SNPs in LD with rs13273672

Analysis of LD structure at the *GATA4* locus (± 100 kb of *GATA4* RefSeq NM_002052) was performed using HapMapIII

CEU data using HAPLOVIEW.¹³ HapmapIII CEU data were downloaded at ftp://ftp.ncbi.nlm.nih.gov/hapmap/genotypes/2009-01_phaseIII/plink_format/hapmap3_r2_b36_fwd.qc.poly.tar.bz2. The SNPs in complete LD with rs13273672 were analyzed for possible functionality *in silico*. First, we assessed whether alleles of SNPs in complete LD to rs13273672 influence *GATA4* mRNA level, using expression Quantitative Trait Locus (eQTL) search at <http://eqtl.uchicago.edu/>.^{14,15} Second, we tested whether SNPs in complete LD with rs13273672 lead to an amino-acid exchange in the *GATA4* protein, using dbSNP build 130 annotation (<http://www.ncbi.nlm.nih.gov/projects/SNP/>). As a test for functional constraint on amino-acid exchanges, we attempted to predict the functional constraint at amino-acid positions. We assessed the conservation profile at single amino-acid sites in a partial alignment of *GATA4* in a diverse set of genetic model organisms, of which whole-genome sequence assemblies are available (<http://genome.ucsc.edu>; also see the study by Blanchette *et al.*¹⁶). The Genome-Wide Splice-Site Single Nucleotide Polymorphism Database (<http://variome.kobic.re.kr/ssSNPTarget/>) was used to search SNPs in conserved splice sites.

Results

Analysis of the 15 most promising SNPs of the GWA analysis and follow-up study

One of the 15 SNPs, an intronic SNP in *GATA4* (rs13273672), was associated with relapse to heavy drinking within the twelve weeks of treatment in the PREDICT-study ($P=0.0066$, Table 1). At the end of the 90 days treatment period, 45.7% of patients from the AA group, 53.9% of the

Table 1 Association of the 15 most promising SNPs of the GWA and follow-up analysis with relapse

SNP	P-value ^a	Allele A	Allele B	Frequency A Abstinent	Frequency A Relapsed	Odds ratio Allele B	CI (OR)
rs11640875	0.2760	A	G	0.371	0.332	1.188	0.878–1.606
rs12388359	0.7048	A	C	0.122	0.135	0.892	0.576–1.381
rs13160562	0.8376	A	G	0.282	0.289	0.968	0.705–1.331
rs13273672	0.0066	A	G	0.712	0.617	1.532	1.125–2.086
rs13362120	0.8275	A	G	0.684	0.692	0.966	0.708–1.318
rs1344694	0.7851	A	C	0.333	0.343	0.956	0.691–1.323
rs1487814	0.5552	A	G	0.562	0.583	0.916	0.684–1.226
rs1614972	0.1423	A	G	0.315	0.262	1.299	0.939–1.797
rs1864982	0.3677	A	C	0.151	0.175	0.836	0.566–1.235
rs36563	0.2784	A	C	0.201	0.171	1.221	0.843–1.767
rs6902771	0.9352	A	G	0.468	0.465	1.013	0.754–1.360
rs705648	0.1392	A	G	0.758	0.710	1.283	0.925–1.780
rs7138291	0.3299	A	G	0.848	0.872	0.818	0.535–1.251
rs729302	0.6601	A	C	0.672	0.657	1.070	0.789–1.452
rs7590720	0.2676	A	G	0.720	0.682	1.199	0.872–1.650

Abbreviations: CI, confidence interval, GWA, genome-wide association; OR, odds ratio; SNP, single nucleotide polymorphism.

^aCochran–Armitage test for trend.

Bold: rs13273672, an intronic SNP in *GATA4*, a transcription factor of the atrial natriuretic peptide (ANP).

patients from the AG group and 69.0% of the patients from the GG group had relapsed to heavy drinking.

Although this effect did not reach statistical significance when compared to a conservative, Bonferroni adjusted alpha level of .0033, it may be regarded as a statistical trend. Considering that this SNP had been associated with alcohol dependence in the GWA analysis on a nominal significance level (2.18×10^{-3}) and that it was taken forward to the follow-up study due to convergent findings from our animal studies, we believe that further analyses of this SNP's link to alcohol dependence are justified for our exploratory purposes while no further analyses were conducted on the remaining 14 SNPs. Naturally, any resulting findings may have to be considered preliminary.

Genotype distribution of rs13273672 did not deviate from Hardy Weinberg Equilibrium (HWE) expectations ($P=1.0$). Of 369 genotyped subjects, 162 were homozygous for the A allele, 42 for the G allele and 165 patients were genotyped as heterozygous. The link between rs13273672 and alcohol dependence was further supported by a survival analysis using information about time to relapse to heavy drinking (logrank test: $\chi^2(\text{degree of freedom}=2)=8.48$; $P=0.014$, Figure 1). As indicated by the survival curves in Figure 1, patients in the GG group relapsed to heavy drinking earlier (Mean survival time (days)=48.67, s.e.=5.31, CI [38.26–59.07]) than patients in the other two genotype groups (AA: Mean survival time (days)=63.22; s.e.=2.66; CI[58.01–68.43]; AG: Mean survival time (days)=59.93, s.e.=2.54, CI[54.95–64.91]) and were more likely to experience a relapse to heavy drinking within the three-month pharmacological treatment period.

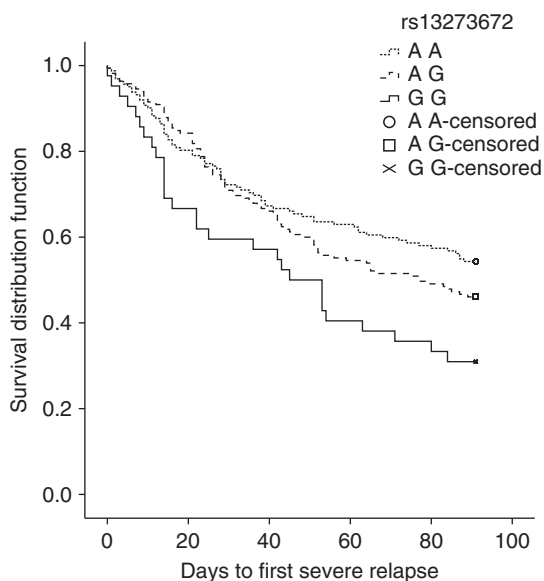


Figure 1 Kaplan–Meier survival curves: time to relapse to heavy drinking within 90 days of treatment as a function of *GATA4* (gene for GATA-binding protein 4) single nucleotide polymorphism (SNP) rs13273672 genotype group (logrank test: $P=0.014$; $N(\text{AA})=162$, $N(\text{AG})=164$; $N(\text{GG})=42$).

Search for potential functional relevance of rs13273672 and SNP in complete LD with rs13273672 in silico

To date, no evidence for functional relevance of G/A exchange in rs13273672 has been described. The analysis of the LD structure within a ± 100 kb region of *GATA4* (NM_002052) RefSeq boundaries, showed that only 16 SNPs reached complete LD ($D'=1$) (Supplementary figure 1). 15 of the 16 SNPs are concentrated within 16 kb up- and downstream of rs13273672 (Supplementary table 1, supplementary figure 1). An exception is rs1065712, which is 90 kbp downstream of rs13273672. The analyses of eQTLs at the *GATA4* locus (defined as the *GATA4* reference sequence ± 100000 bp) did not detect a HapMap Phase III SNP in complete LD with rs13273672, which constitutes an eQTL.

In search for amino-acid exchanges, we observed rs13273672 in complete LD ($D'=1.0$) with a common amino-acid exchange at position 377 of the *GATA4* protein (rs3729856), which has a moderate population frequency of 11% in the HapMapIII CEU sample. However, comparative analysis of this single amino-acid site with that of diverse genetic model organisms of which whole-genome sequences are available, suggests no strong functional constraint on this position (Supplementary figure 2). This is in congruence with the recent experimental observation that S377G (rs3729856) does not cause transcriptional deficits of the *GATA4* protein in transactivation assays.¹⁷ rs13273672 and SNPs in complete LD with rs13273672 were not listed among the SNPs in conserved splice sites in the Genome-Wide Splice-Site Single Nucleotide Polymorphism Database.

Clinical disease characteristics

Given the influence of rs13273672 on time to first relapse to heavy drinking, it was investigated whether individuals from the three genotype groups differed in any clinical disease characteristics using independent-samples *t*-tests. The results showed that the three genotype groups did not differ in the number of fulfilled DSM-IV or ICD-10 criteria of alcohol dependence, onset and duration of alcohol dependence, amount of daily alcohol consumed within a period of 90 days before study onset, severity of alcohol dependence as measured by the alcohol dependence scale¹⁸, alcohol craving as assessed by the alcohol urge questionnaire¹⁹, severity of nicotine dependence (Fagerström Test of Nicotine Dependence²⁰) or trait anxiety as assessed by the state trait inventory²¹, all P -values >0.05 . Data not shown.

Subgroup analysis of treatment groups

To further investigate the specific effect of SNP rs13273672 on treatment outcome, we performed trend tests within the three treatment groups receiving acamprostate, naltrexone or placebo. The distribution of participants across these subgroups was as follows: from the AA group, 35, 60 and 67 patients received placebo, acamprostate and naltrexone treatment, respectively, whereas in the AG group the distribution was 30, 65 and 70 and in the GG group the distribution was 9, 22 and 11 for placebo, acamprostate and naltrexone, respectively. The *GATA4* (rs13273672) gene was significantly associated with relapse within the 90-day

Table 2 Association tests between *GATA4* SNP rs13273672 and abstinence proportion after 90 days of pharmacological treatment

	Group size ^a	P-value ^b	Allele A	Allele B	Frequency A Abstinent	Frequency A Relapsed	Odds ratio	CI (OR)
Acamprosate	147	0.0013	A	G	0.725	0.539	2.255	1.385–3.670
Naltrexone	148	0.3006	A	G	0.717	0.665	1.281	0.780–2.105
Placebo	74	1.0000	A	G	0.676	0.676	1.000	0.502–1.990

Abbreviations: CI, confidence interval; OR, odds ratio; SNP, single nucleotide polymorphism.

^aEffective sample size after excluding missing values.

^bCochran–Armitage test for trend.

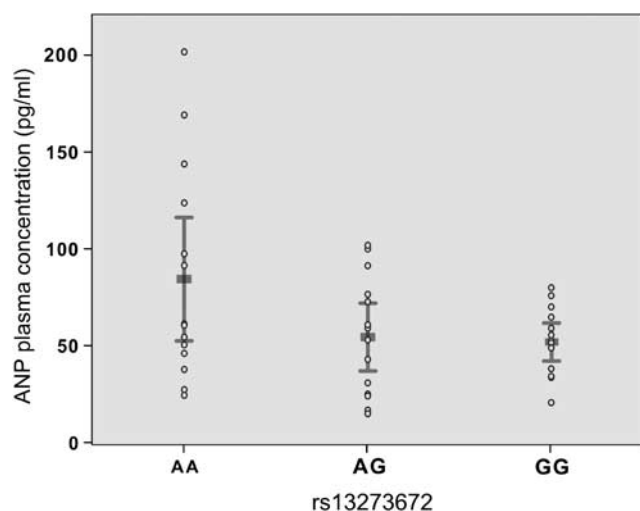


Figure 2 Atrial natriuretic peptide (ANP) plasma concentration as a function of *GATA4* (gene for GATA-binding protein 4) single nucleotide polymorphism (SNP) rs13273672 (modified Levene test of variance differences: $P=0.003$; $n=42$).

treatment period in patients treated with acamprosate (P -value = 0.0013), but not in the naltrexone ($P=0.3$) or the placebo group ($P=1.0$; Table 2).

Analysis of ANP plasma concentration as a function of *GATA4*

As *GATA4* is involved in the regulation of ANP gene expression,⁵ we further assessed the effect of rs13273672 on ANP plasma concentration. A total of 42 subjects, 14 of each genotype matched for age, sex and age of dependence onset, were included in this exploratory analysis. Distribution of ANP plasma concentration differed significantly between the three genotypes showing an increased variance in the AA group (modified Levene test: $P=0.003$; Figure 2). This group also showed the highest ANP concentrations (84.1 ± 14.8 pg per ml (mean \pm s.e.m.)) compared with the heterozygous (54.1 ± 8.1 pg per ml) and the GG group (51.6 ± 4.5 pg per ml; Figure 2).

Discussion

It may seem plausible that genes linked to the development of alcohol dependence (that is, a condition characterized by continuous, compulsive use of alcohol despite the wish to

stop or reduce consumption) also influence abstinence and relapse behavior. However, research findings from the field of nicotine dependence clearly indicate that genes relevant to the development of addictive smoking behaviors may not necessarily also influence the probability of cessation once a level of heavy smoking has been reached (for example, see the study by Breitling *et al.*⁸). Overall, the results of the current study are consistent with this observation. Genetic variations that are associated with alcohol dependence are not necessarily associated with the risk of relapse. Nevertheless, there seem to be exceptions. The main result of our investigation suggests that SNP rs13273672, which is located intronically in the *GATA4* gene and which has been linked to alcohol dependence in a recent GWA and follow-up study is indeed associated with abstinence and abstinence duration after detoxification treatment in the sample investigated. Moreover, this SNP also seems to modulate the response to pharmacological relapse prevention treatment with acamprosate in a gene-dose dependent way. This modulatory effect was not observed in patients treated with naltrexone or placebo.

The *GATA4* protein is a member of a zinc finger family of DNA-binding proteins, which recognize the 'GATA' motif, a consensus sequence in the promoters of several genes.^{22–24} The role of *GATA4* in relapse behavior of alcohol-dependent individuals deserves closer attention, as *GATA4* represents a transcription factor regulating the transcription of the ANP⁵, a peptide repeatedly shown to be involved in the pathophysiology of withdrawal and relapse.⁷ Atrial natriuretic peptide is secreted in cardiac atria and is involved in regulating blood pressure and volume through its natriuretic and diuretic properties.²⁵ However, ANP-binding sites (natriuretic peptide receptor-A, -B and -C) are also located in various regions of the brain, including the hypothalamus, the pituitary gland, the adrenal medulla and the amygdala.²⁶ These findings led to the proposition that its role may reach beyond the central regulation of cardiovascular parameters. Indeed, ANP was found to be a relevant component in the neuroendocrine regulation of stress. It attenuates the stress response by inhibiting the HPA system at the hypothalamic, pituitary, and adrenocortical level.^{27–29} Moreover, a specific role of ANP in counteracting centrally mediated anxiogenic effects of corticotropin-releasing hormone and ANP's influence on the regulation of affective and anxiety symptoms are currently being discussed.^{30–33} In mice, intracerebroventricular

injections of ANP were found to attenuate hyperexcitability during alcohol withdrawal, whereas injections of an antiserum against ANP intensified it.⁶ Correspondingly, human studies reported a dysregulation of ANP plasma concentration during alcohol withdrawal that contributed to symptoms of protracted withdrawal. Specifically, detoxified patients with decreased ANP plasma concentrations during alcohol withdrawal suffered from more intense and frequent craving, as well as from higher anxiety levels.³⁴ In addition, ANP mRNA expression was significantly elevated in alcoholic patients, whereas promoter-related DNA methylation of ANP was significantly decreased. Furthermore, promoter-related DNA methylation of ANP was significantly correlated with the extent of craving.³⁵ The data presented here suggest that ANP plasma concentration is influenced by a variant in *GATA4* with the AA/AG genotype of SNP rs13273672. In particular, the reduced variability of ANP in carriers of at least one G allele, observed in this study, may be indicative of a diminished metabolic responsiveness and consequently a reduced ANP synthesis.

Beyond the observed relationship between *GATA4* and relapse in alcoholism, suggested here to be partially mediated by ANP, our explorative analysis showed an association between this SNP and the response to pharmacological relapse prevention with acamprosate in a gene dose-dependent manner. Acamprosate was repeatedly shown to be efficacious in relapse prevention treatment of alcoholism.^{36,37} It exerts its actions mainly through glutamatergic mechanisms but the exact mode of action on the receptor level remains unclear.^{9,38} Atrial natriuretic peptide also influences glutamate-mediated effects. For instance, intracerebroventricular administration of ANP in alcohol-dependent mice resulted in an attenuation of glutamate-mediated seizures, whereas administration of an antiserum against ANP potentiated seizure activity.⁶ Therefore, we hypothesize that *GATA4*-dependent ANP activity may impact on the glutamate system, the main target system of acamprosate's action. Alternatively, a common end point of *GATA4*-dependent ANP activity and acamprosate action is the activity of the HPA system. Acamprosate treatment is accompanied by an increase of beta-endorphin plasma concentration in rats and humans,^{39,40} mediating inhibitory effects on the HPA axis at the corticotropin-releasing hormone and adrenocorticotrophic hormone level. It was suggested that opioids have a significant role in controlling ANP release. In fact, circulating levels of beta-endorphin increase rapidly after alcohol injection with a time course similar to that of ANP,⁶ and a high, positive correlation between alcohol-induced changes in plasma ANP and beta-endorphin was observed.⁴¹ Hence, acamprosate and *GATA4* may interact in counter-regulating corticotropin-releasing hormone effects leading to a dampening of the stress response.

This study has a number of limitations. First, the rs13273672 SNP is located intronically in the 3'-portion of the *GATA4* gene, and is therefore not causing an amino-acid substitution in the encoded *GATA4* protein. Nevertheless a regulatory effect on gene functioning, for example, by alteration of the level of transcription due to impaired

binding of regulatory elements or by influences on mRNA splicing cannot be excluded. However, *in silico* analysis did not reveal any functional effect of this SNP (Supplementary Information). Alternatively, the observed effects could be due to other variants in LD with rs13273672. Our analyses of SNPs in HapMap III CEU data revealed no SNP in complete LD ($D' = 1.0$) with rs13273672 that was predicted to have possible functionality (for details see Supplementary Information). Although we could not find any functional variant in close LD with rs13273672, it is still possible that this region hosts hitherto undetected causal variants.

Thus, re-sequencing is needed to identify causal variants that influence ANP expression directly or indirectly or to determine whether this variant acts through other pathways that have not been investigated yet. Second, the data suggestive of a gene effect on ANP plasma concentration provided by our investigation can only be considered preliminary. Future gene-expression and replication studies should examine the interactions between ANP plasma concentration, HPA axis activity and relapse-associated phenotypes more directly. Third, our findings have to be regarded preliminary because they represent an explorative approach defining the functional relevance of a SNP shown to be associated with the phenotype of alcohol dependence.

In conclusion, our results provide evidence for a link between the tested genetic variation in *GATA4* and relapse behavior and for a link between this SNP and treatment response to acamprosate. On the basis of data from the first GWA and its follow-up study, which applied individual genotyping to alcohol dependence, the present investigation is, to the best of our knowledge, the first to provide data suggestive of a genotype-modulated treatment response to acamprosate and may thus represent a valuable step toward personalized medical treatment.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

After the manuscript had been accepted, an independent GWAS on alcohol dependence confirmed the association finding of the *GATA4* rs13273672 risk allele (Edenberg HJ, Koller DL, Xuei X, Wetherill L, McClintick JN, Almasy L *et al*. Genome-wide Association Study of Alcohol Dependence Implicates a Region on Chromosome 11. *Alc Clin Exp Res* 2010; **34**: 840–852).

We thank Iris Reinhard from the Department of Biostatistics of the Central Institute of Mental Health for her statistical support. The study was supported by the National Genome Research Network (NGFN) and the German Federal Ministry of Education and Research (BMBF; FKZ 01GS0117/NGFN, 01GS8152/NGFN-plus, FKZ EB 01011300 and 01EB0410), and by the federal state of Baden-Württemberg (DNA-bank addiction). All authors declare that they have no conflict of interest, including financial interests and relationships and affiliations relevant to the subject of the manuscript. FK, MR and SHW had full access to all the data in the study and take full responsibility for the integrity of the data and the accuracy of data analysis.

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