Alcohol and Alcoholism, 2018, 53(4) 361–367
doi: 10.1093/alcalc/agx120
Advance Access Publication Date: 5 February 2018
Article

OXFORD

Article

Association Analysis Between Genetic Variation in GATA Binding Protein 4 (*GATA4*) and Alcohol Use Disorder

Kelsey L. Mauro[1], Sarah G. Helton[1], Dan B. Rosoff[1], Audrey Luo[1], Melanie Schwandt[2], Jeesun Jung[3], Jisoo Lee[1], Christine Muench[1], and Falk W. Lohoff[1,*]

[1]Section on Clinical Genomics and Experimental Therapeutics, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, 10 Center Drive (10CRC), Bethesda, MD 20892, USA, [2]Office of the Clinical Director, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, 10 Center Drive (10CRC), Bethesda, MD 20892, USA, and [3]Epidemiology & Biometry Branch, Division of Epidemiology and Prevention Research, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, 5635 Fishers Ln., Rockville, MD 20852, USA

*Corresponding author: Section on Clinical Genomics and Experimental Therapeutics (CGET), National Institute on Alcohol Abuse and Alcoholism (NIAAA), National Institutes of Health, 10 Center Drive (10CRC/2-2352), Bethesda, MD 20892-1540, USA. Tel: +1-301-827-1542; E-mail: falk.lohoff@nih.gov

Received 20 September 2017; Revised 30 November 2017; Editorial Decision 14 December 2017; Accepted 20 December 2017

Abstract

Aims: Previous genetic association studies have shown that variation in the *GATA4* gene encoding the GATA binding protein 4, a binding protein that binds to the ANA sequence GATA, increase susceptibility for alcohol use disorder (AUD). In this study, we aimed to replicate those findings in an independent sample and analyze their association with anxiety.

Methods: Overall, 1044 individuals with AUD [534 European American (EA), 510 African Americans (AA)] and 645 controls [413 EA, 232 AA] were genotyped using 34 markers. Genotype and allele frequencies were compared between cases and controls using chi-square analysis. Other phenotype data were analyzed for possible associations with *GATA4* single-nucleotide polymorphisms (SNPs) in individuals with AUD.

Results: Rs6601604 was nominally significantly associated with AUD in EA, and 3 SNPs (rs6990313, rs11250159 and rs17153694) showed trend-level significance ($P < 0.10$) in AA. However, none of the SNPs were significant after correcting for multiple testing. Haplotype analysis of the 34 SNPs did not find a significant association between haplotype blocks and AUD diagnosis after correcting for multiple testing. From the phenotype analysis, anxiety was associated with *GATA4* SNP rs10112596 among the AA group with AUD after a correction for multiple testing.

Conclusions: Although previous studies have shown a relationship between variants of the *GATA4* gene and a diagnosis of AUD, we did not replicate these findings in our sample. Additional studies of variation in this gene are needed to elucidate whether polymorphisms of the *GATA4* gene are associated with AUD and other alcohol-related phenotypes.

Short Summary: *GATA4* variants were not associated with AUD in either the European ancestry or African ancestry groups after correcting for multiple comparisons. Rs10112596 demonstrated a significant relationship with an anxiety measure among the African ancestry group with AUD.

INTRODUCTION

The prevalence, individual health risks and societal costs of excessive alcohol use demonstrate the importance of identifying underlying causes of pathological alcohol consumption and developing novel treatment approaches (Bouchery *et al.*, 2011; Stahre *et al.*, 2014; Grant *et al.*, 2015). Genetic factors account for ~40–60% of the variance in risk of developing alcohol use disorder (AUD) (Rietschel and Treutlein, 2013; Tawa *et al.*, 2016); however, AUD is a complex disorder, with many factors contributing to its onset and maintenance. Identification of the underlying genetic risk variants will further our understanding of the disorder's neurobiology and may direct the development of novel individualized (i.e. pharmacogenetic) treatment options for AUD.

Recent case-control genome-wide association studies (GWAS) implicate GATA binding protein 4 (*GATA4*), a gene located on chromosome 8, in associations with vulnerability to AUD diagnosis (Treutlein *et al.*, 2009; Edenberg *et al.*, 2010). While a previous candidate gene study found a significant association between *GATA4* and AUD using gene-level testing (Karpyak *et al.*, 2014), a more recent study by Degenhardt *et al.* (2016) failed to show an association between rare *GATA4* variants and AUD. However, it should be noted that Degenhardt *et al.* (2016) attempted to identify only rare risk-associated variants, which precluded them from identifying likely protective variants in *GATA4*.

The *GATA4* gene encodes transcription factor GATA4, which regulates the expression of the atrial natriuretic peptide (ANP), among other proteins (McBride and Nemer, 2001). Importantly, GATA4 protein is expressed throughout cells in the central nervous system (CNS). Reduced ANP expression in the CNS is associated with the dysregulation of stress and anxiety mechanisms in the brain, suggesting a possible link between ANP and AUD (Jorde *et al.*, 2014). ANP also influences hypothalamic–pituitary–adrenal (HPA) axis functioning, as well as amygdala activation, further supporting the relationship between ANP and AUD-related phenotypes (McBride and Nemer, 2001). Clinically, post-detoxification patients with AUD and decreased ANP plasma levels report increased craving and anxiety levels compared to both detoxified patients with AUD and higher ANP plasma levels, as well as controls (Kiefer *et al.*, 2002). GATA4 also acts as a transcription factor for brain natriuretic peptide (BNP), a peptide involved in the regulation of the cardiovascular system. Interestingly, BNP is also involved in stress responses when found in the CNS (Amir *et al.*, 2010). One study demonstrated a relationship between *GATA4* binding site methylation and BNP expression among alcohol-dependent patients experiencing alcohol withdrawal (Glahn *et al.*, 2016).

Previous GWAS highlight an association between the specific intronic single-nucleotide polymorphism (SNP) rs13273672 in the *GATA4* gene and AUD-related phenotypes (Kiefer *et al.*, 2011; Jorde *et al.*, 2014). In a randomized, double-blind, placebo-controlled study, Kiefer *et al.* (2011) showed that alcohol-dependent individuals with the rs13273672 G allele had a decreased time to relapse following Acamprosate treatment compared to A allele carriers with AUD. Furthermore, the G allele of this SNP was predictive of a significant decrease in variance in ANP plasma concentration compared to the A allele. Acamprosate is an FDA-approved pharmacological intervention for AUD that decreases cravings to reduce relapse risk; it is thought to primarily work through the glutamatergic system, although the exact mechanism of action remains unclear (Kiefer *et al.*, 2011). Further research identified the AA genotype as associated with stronger alcohol cue-induced amygdala activation, and this association was predictive of a lower relapse risk (Jorde *et al.*, 2014). Zois *et al.* (2016) expanded on this work by identifying an interaction between *GATA4* genotype and gray matter volume on relapse risk, such that the AA genotype group showed an association between greater gray matter and a reduced relapse risk. This provides further support for the possible protective nature of the AA genotype.

Furthermore, AUD has been shown to be associated with mood and anxiety disorders, neuroticism and alcohol withdrawal (Regier *et al.*, 1990; Malouff *et al.*, 2007; Becker and Mulholland, 2014), all of which may play a role in genetic predisposition to AUD (Tawa *et al.*, 2016). In particular, much research has determined the significant co-occurrence of AUD and anxiety disorders, and it is likely that genetic variation influences this comorbidity (Poikolainen, 2000; Smith and Randall, 2012). According to the common factor model proposed by Smith , a third variable (e.g. genetic susceptibility) explains the presence of both AUD and anxiety disorders. In line with this model, Merikangas *et al.* (1996) found that relatives of patients with anxiety disorders had an increased risk for alcohol dependence. This result could be partly explained by shared genetic factors influencing susceptibility to AUD and anxiety disorders. Moreover, a review by Kenna *et al.* (2012) highlights more recent research that has found an association between 5-HTTLPR, a 5-HT transporter polymorphism, and both alcohol dependence and anxiety symptoms. Given the high degree of heritability of AUD and frequent comorbid occurrence of anxiety symptoms, identifying genetic risk factors that contribute to their shared pathophysiology may improve our understanding of comorbid AUD and anxiety, as well as inform the development of pharmacological treatments. Therefore, the present study aimed to explore associations between genetic variation in *GATA4* and anxiety, as assessed by the Brief Scale for Anxiety.

In summary, previous studies indicate a possible association between variation in the *GATA4* gene and AUD. However, the underlying mechanisms of this relationship are still relatively unknown and poorly understood. Therefore, additional studies of SNPs within *GATA4* and their association with AUD-related phenotypes are needed. In this case-control study, we sought to (a) replicate findings associating variants in *GATA4* with increased susceptibility to AUD and (b) determine associations between *GATA4* variants and alcohol-related clinical phenotypes, specifically anxiety, as assessed by the Brief Scale for Anxiety. Identifying genetic variants associated with AUD and related clinical phenotypes could be used to identify individuals at risk of developing AUD. Ultimately, this could inform the development of more targeted pharmacological prevention and treatment approaches for AUD.

MATERIALS AND METHODS

Participants

This study was approved by the Institutional Review Board at the National Institutes of Health (NIH). All participants provided written informed consent and permission to use collected samples. Out of 1778 individuals with collected samples, 1044 individuals with AUD [534 European Americans (EA), 510 African Americans (AA)] and 645 controls [413 EA and 232 AA] took part in this study. The 89 missing participants were excluded because they did not have a completed SCID diagnosis. Study participants were recruited between 2005 and 2016 from the inpatient unit and outpatient clinic

of the Laboratory of Clinical and Translational Studies at the National Institute on Alcohol Abuse and Alcoholism (NIAAA), NIH (Bethesda, MD). Participants were recruited from three screening protocols, all of which excluded those under 18 years of age. Two of the screening protocols included only those in good health without major medical problems, and excluded individuals that were under court-mandated or involuntary treatment. The third protocol excluded prisoners, as well as pregnant women. Alcohol-dependent subjects were diagnosed with alcohol dependence according to the *Diagnostic and Statistical Manuel for Mental Disorders, 4th edn, Text-revised* (DSM-IV-TR) (American Psychiatric Association, 2000). Participants were diagnosed using the Structured Clinical Interview (SCID-I) for DSM-IV-TR (First, *et al.*, 2002). Given the overlap between the DSM-IV alcohol dependence criteria and the *Diagnostic and Statistical Manual of Mental Disorders, 5th edn* (DSM-5) (American Psychiatric Association, 2013) AUD criteria, all participants also met criteria for AUD; however, a separate clinical interview was not conducted. Informed consent was obtained from all subjects who participated in accordance with the Declaration of Helsinki.

Genotyping and SNP selection

Large-scale genotyping was performed at the NIAAA Laboratory of Neurogenetics using the Illumina OmniExpress BeadChip (Illumina, San Diego, CA). Data for all SNPs located within the *GATA4* gene that were genotyped on the array were extracted using PLINK version 1.07 (Purcell *et al.*, 2007) (http://pngu.mgh.harvard.edu/purcell/plink/), based on start and end base pair positions for the gene located on chromosome 8 (11561716, 1161750; GRCh37/hg19 assembly). This procedure resulted in genotype data for 34 SNPs. Ancestry informative markers (AIMs; $n = 2500$) were also extracted from the Illumina array to calculate ancestral proportions for all study participants. Using methods described previously for an AIM panel including 186 markers (Hodgkinson *et al.*, 2008), which were not available for the current data set, the ancestry assessment identified six ethnic factors (Africa, Europe, Asia, Far East Asia, Oceania and Americas). An analysis of the 34 SNPs among the full sample ($n = 1778$) found that all were in Hardy–Weinberg equilibrium (HWE) except rs12550668 ($P < 0.005$) and rs3729856 ($P < 0.033$) in the EA group. In the AA group, all SNPs were in HWE except rs6601604 ($P < 0.03$), rs804280 ($P < 0.004$) and rs867858 ($P < 0.003$).The same analysis found that rs10105409 in the EA group and rs13275657, rs17153747, rs3729856, rs804290 and rs11785481 in the AA group all had minor allele frequencies (MAF) < 5%. All other SNPs had a MAF > 5%.

Analysis

Allele frequencies for each SNP were determined using PLINK for the sample as a whole, and then separately for subjects of European and African ancestry (based on self-report). Due to multiple differences in allele frequency across the 34 SNPs, subsequent analyses were conducted separately in each group. Single marker association analyses were conducted using frequency comparison by chi-square test, which is the standard case-control method in PLINK, with adjustment for multiple comparisons performed using the Benjamini–Hochberg method for false discovery rate (FDR) (Benjamini and Hochberg, 1995). The threshold for FDR was set at $q = 0.05$. Haplotype blocks were determined using HaploView software (Barrett *et al.*, 2005), with haplotype blocks defined using the default D'/LOD method. Haplotype association tests using these defined blocks were conducted in PLINK, and were corrected for multiple comparisons using permutation tests (5000 permutations).

Participants also completed a variety of clinical assessments, including the Alcohol Dependence Scale (ADS; Skinner and Allen, 1982), Montgomery Asberg Depression Rating Scale (MADRS; Montgomery and Asberg, 1979), Brief Scale for Anxiety (BSA; Tyrer *et al.*, 1984), State-Trait Anxiety Inventory (STAI; Spielberger *et al.*, 1970), Clinical Institute Withdrawal Assessment for Alcohol (CIWA; Sullivan *et al.*, 1989) and NEO-PI-R (Costa and McCrae, 2002). Sample sizes for these assessments are inconsistent due to missing data, particularly among the control group who were not administered these assessments until later in the study's recruitment. Single marker association and haplotype analyses were conducted for these continuous outcomes using linear regression models in PLINK. These analyses controlled for age, gender, and African and European ancestry via the AIMS scores for Africa and Europe, based on research showing age, gender and ethnicity differences in alcohol consumption and its related consequences (Delker *et al.* 2016).

RESULTS

Table 1 shows the demographic information of participants, as well as differences in the clinical assessments between groups. As expected, AUD participants had significantly greater scores in all alcohol-related phenotype measures when compared to controls in both the EA and AA group. To analyze the association between the 34 *GATA4* SNPs and AUD diagnosis, single marker association analyses were conducted using frequency comparison by chi-square test with adjustment for multiple comparisons using the Benjamini–Hochberg method for FDR. Results revealed that one SNP (rs6601604) was nominally significantly associated with AUD in the

Table 1. Demographic and clinical assessment information

	European ancestry			African ancestry		
	AUD ($n = 534$)	Controls ($n = 413$)	*P*-value[a]	AUD ($n = 510$)	Controls ($n = 232$)	*P*-value[a]
Gender count (female)	166 (31.1%)	175 (42.4%)	0.0003	140 (27.5%)	107 (46.1%)	<0.0001
Mean age (SD)	42.5 (11.4)	32.0 (12.1)	<0.0001	43.2 (10.1)	35.6 (11.0)	<0.0001
Mean ADS score (SD)	21.8 (8.3) [$n = 451$]	2.1 (4.1) [$n = 95$]	<0.0001	17.7 (8.6) [$n = 345$]	1.3 (3.9) [$n = 94$]	<0.0001
Mean MADRS score (SD)	15.3 (9.7) [$n = 444$]	1.5 (3.5) [$n = 166$]	<0.0001	11.2 (9.4) [$n = 441$]	1.2 (3.1) [$n = 155$]	<0.0001
Mean BSA score (SD)	11.1 (7.0) [$n = 446$]	1.3 (2.6) [$n = 166$]	<0.0001	8.7 (7.2) [$n = 441$]	1.1 (2.6) [$n = 155$]	<0.0001
Mean STAI score (SD)	43.2 (13.6) [$n = 199$]	33.6 (11.5) [$n = 185$]	<0.0001	41.1 (12.4) [$n = 235$]	32.4 (11.9) [$n = 145$]	<0.0001
Mean neuroticism score (SD)	56.3 (11.5) [$n = 483$]	44.8 (10.0) [$n = 377$]	<0.0001	54.6 (9.7) [$n = 444$]	44.9 (8.4) [$n = 202$]	<0.0001

[a]Chi-square test for gender; *t*-test for all remaining continuous variables.

EA group ($P = 0.036$). However, this SNP was not significant after adjusting for multiple comparisons (Table 2). Three SNPs (rs6990313, rs11250159 and rs17153694) trended towards a significant association with AUD in the AA group ($Ps \leq 0.065$) (Table 3), but these SNPs failed to reach trend-level significance after adjusting for multiple comparisons. The a priori SNP of interest, rs13273672, was not significantly associated with AUD for either ancestry group in our sample.

In addition to tests of single SNP associations, we ran haplotype analyses of the 34 SNPs. Interestingly, the haplotype structure differed in the EA and AA populations. There were nine haplotype blocks in the EA group (Supplementary Fig. S1), and seven haplotype bocks in the AA group (Supplementary Fig. S2).

Two haplotype blocks (Blocks 1 and 5) were nominally significantly related to AUD in the EA group ($P = 0.037$, $P = 0.015$, respectively). Block 1 included rs6601604 (Supplementary Table S1), which was nominally significantly associated with AUD in the single SNP association (Table 2). One block (Block 9) reached trend-level significance in this group ($P = 0.087$) (Supplementary Table S1). Two haplotype blocks (Block 2 and Block 3) trended towards a significant relationship with AUD in the AA group ($Ps \leq 0.068$). Block 3 contained both rs11250159 and rs17153694 (Supplementary Table S2), both of which trended towards significance in the single SNP association (Table 3). There was no significant association between haplotype blocks and AUD diagnosis after correction for multiple testing.

Further analyses of continuous phenotype outcomes co-varied for age, gender, and African and European ancestry. These analyses showed no significant relationships that survived multiple comparisons between the *GATA4* SNPs and scores on the ADS, MADRS, STAI, CIWA or NEO Neuroticism (data not shown). When analyzing only those with current AUD, one phenotype, anxiety, as measured by the BSA was significantly associated with *GATA4* SNP rs10112596 when adjusting for multiple comparisons ($P = 0.032$) in the AA ancestry only (Table 4). This SNP was not in a haplotype block.

DISCUSSION

Previous studies have found evidence that implicates the *GATA4* gene in susceptibility to alcohol dependence (Treutlein *et al.*, 2009; Edenberg *et al.*, 2010; Karpyak *et al.*, 2014). In particular, the SNP rs13273672 has been found to be related to variance in ANP

Table 2. Associations between *GATA4* gene SNPs and AUD in EA sample[a]

SNP	A1/A2[b]	MAF cases[c]	MAF controls	Chi square	Odds ratio	*P*-value	FDR
rs6990313	A/C	0.10	0.09	1.37	1.21	0.243	0.836
rs10105409	G/A	0.01	0.00	0.05	1.16	0.818	0.904
rs6601604	A/G	0.29	0.34	4.42	0.81	0.036*	0.836
rs10112596	A/G	0.17	0.19	1.33	0.87	0.248	0.836
rs12550668	A/G	0.40	0.43	1.74	0.88	0.188	0.836
rs2898292	G/A	0.10	0.10	0.00	1.00	0.995	0.995
rs4840579	G/A	0.39	0.40	0.28	0.95	0.597	0.836
rs11250159	A/C	0.08	0.09	0.65	0.87	0.421	0.836
rs17153694	A/G	0.07	0.08	0.18	0.93	0.670	0.836
rs17153698	A/G	0.16	0.15	0.24	1.06	0.625	0.836
rs6983129	C/A	0.47	0.48	0.59	0.93	0.444	0.836
rs2898295	A/G	0.50	0.47	1.06	1.10	0.304	0.836
rs11250163	C/A	0.47	0.45	0.69	1.08	0.407	0.836
rs13275657	A/G	0.19	0.20	0.64	0.91	0.423	0.836
rs2029969	G/A	0.38	0.36	0.53	1.07	0.465	0.836
rs2173117	A/C	0.33	0.30	1.78	1.14	0.182	0.836
rs3779664	A/G	0.14	0.16	1.08	0.87	0.299	0.836
rs3735814	A/G	0.48	0.49	0.18	0.96	0.671	0.836
rs2740434	A/G	0.33	0.34	0.13	0.97	0.720	0.844
rs2645399	A/G	0.34	0.36	0.49	0.93	0.486	0.836
rs11784693	A/G	0.29	0.32	1.44	0.89	0.231	0.836
rs804283	G/A	0.29	0.31	1.24	0.89	0.265	0.836
rs17153747	G/A	0.13	0.12	0.75	1.13	0.386	0.836
rs804282	C/A	0.45	0.46	0.40	0.94	0.529	0.836
rs13264774	A/G	0.15	0.15	0.02	1.02	0.880	0.935
rs13273672	G/A	0.30	0.29	0.26	1.05	0.610	0.836
rs804280	C/A	0.43	0.44	0.32	0.95	0.574	0.836
rs3729856	G/A	0.14	0.14	0.16	1.06	0.689	0.836
rs867858	C/A	0.31	0.31	0.05	1.02	0.824	0.904
rs1062219	A/G	0.45	0.46	0.20	0.96	0.655	0.836
rs804290	A/G	0.24	0.22	1.31	1.13	0.253	0.836
rs11785481	A/G	0.14	0.15	0.26	0.93	0.607	0.836
rs12458	T/A	0.33	0.31	0.36	1.06	0.548	0.836
rs3203358	C/G	0.33	0.32	0.01	1.01	0.917	0.944

[a]$N = 534$ cases, 413 controls.
[b]Alleles 1 and 2 refer to minor and major allele, respectively.
[c]MAF = minor allele frequency.
*$P < 0.05$.

Table 3. Associations between *GATA4* gene SNPs and AUD in AA sample[a]

SNP	A1/A2[b]	MAF cases[c]	MAF controls	Chi square	Odds ratio	*P*-value	FDR
rs6990313	A/C	0.28	0.32	3.39	0.80	0.065	0.742
rs10105409	G/A	0.17	0.19	0.96	0.87	0.327	0.930
rs6601604	A/G	0.43	0.41	0.62	1.09	0.431	0.930
rs10112596	A/G	0.16	0.14	0.86	1.16	0.353	0.930
rs12550668	G/A	0.11	0.10	0.66	1.16	0.417	0.930
rs2898292	G/A	0.30	0.27	2.06	1.20	0.151	0.921
rs4840579	A/G	0.47	0.46	0.40	1.07	0.527	0.930
rs11250159	A/C	0.21	0.17	3.41	1.31	0.065	0.742
rs17153694	A/G	0.09	0.06	3.79	1.54	0.051	0.742
rs17153698	A/G	0.32	0.31	0.29	1.07	0.588	0.930
rs6983129	C/A	0.38	0.35	1.46	1.15	0.226	0.930
rs2898295	A/G	0.39	0.38	0.12	1.04	0.728	0.930
rs11250163	C/A	0.10	0.10	0.02	0.98	0.898	0.930
rs13275657	A/G	0.04	0.03	0.64	1.27	0.425	0.930
rs2029969	G/A	0.24	0.24	0.01	0.99	0.927	0.930
rs2173117	A/C	0.24	0.23	0.34	1.08	0.559	0.930
rs3779664	A/G	0.06	0.06	0.13	1.09	0.721	0.930
rs3735814	A/G	0.49	0.49	0.06	1.03	0.802	0.930
rs2740434	A/G	0.31	0.31	0.01	1.01	0.930	0.930
rs2645399	A/G	0.48	0.51	0.69	0.91	0.408	0.930
rs11784693	A/G	0.22	0.21	0.12	1.05	0.726	0.930
rs804283	G/A	0.13	0.11	1.72	1.26	0.190	0.921
rs17153747	G/A	0.05	0.04	0.27	1.15	0.605	0.930
rs804282	C/A	0.46	0.48	0.18	0.95	0.671	0.930
rs13264774	A/G	0.22	0.26	1.82	0.84	0.177	0.921
rs13273672	G/A	0.37	0.38	0.20	0.95	0.657	0.930
rs804280	C/A	0.42	0.41	0.20	1.05	0.655	0.930
rs3729856	G/A	0.02	0.02	0.63	0.74	0.428	0.930
rs867858	C/A	0.23	0.23	0.06	0.97	0.810	0.930
rs1062219	A/G	0.17	0.17	0.03	1.03	0.867	0.930
rs804290	A/G	0.05	0.03	2.20	1.54	0.138	0.921
rs11785481	A/G	0.03	0.03	0.03	0.94	0.852	0.930
rs12458	T/A	0.39	0.39	0.02	0.98	0.892	0.930
rs3203358	C/G	0.06	0.06	0.19	1.11	0.667	0.930

[a]N = 510 cases, 232 controls.
[b]Alleles 1 and 2 refer to minor and major allele, respectively.
[c]MAF = minor allele frequency.

expression, alcohol-induced cue reactivity and relapse risk (Kiefer *et al.*, 2011; Jorde *et al.*, 2014; Zois *et al.*, 2016).

This case-control study aimed to replicate previous GWAS and candidate gene studies relating *GATA4* and SNP rs13273672 with AUD and alcohol-related phenotypes. Although previous studies have shown a relationship between variants of this gene and a diagnosis of AUD, we did not replicate these findings in our sample.

There are several explanations for these discrepant results. First, as our study consisted of a relatively small sample size, we may have lacked adequate power to detect small effects, which is a limitation of the current study. This limitation might be particularly relevant given the number of SNPs that did not have a MAF > 5%. While Karpyak *et al.* (2014) used a sample of over 800 AD cases, we were limited to 534 and 510 AUD cases in the EA and AA subgroups, respectively. Clinical heterogeneity, such as differences in anxiety or participant status, may also account for our inability to replicate past studies. Edenberg *et al.* (2010) and Treutlein *et al.* (2009) used a sample of participants receiving treatment for their alcohol use, while our cohort included both treatment-seeking and non-treatment-seeking individuals. Although all AUD patients in the present study received a diagnosis based on the DSM-IV, it is possible that the two cohorts represent different phenotypes, which may have confounded the analysis. Furthermore, it is likely that multiple genes are involved in AUD, with only their interaction accumulating to account for a significant proportion of the variance. Therefore, additional studies of genetic variation are needed to elucidate whether polymorphisms of the *GATA4* gene interact with other genes to contribute to the genetic risk for AUD and other alcohol-related phenotypes. Given that one SNP in the EA group (rs6601604) and three SNPs in the AA group (rs6990313, rs11250159 and rs17153694) did not survive correction for multiple comparisons, our data indicate a need for further replication studies with larger sample sizes. Karpyak *et al.* (2014) used gene-level testing to identify an association between AUD diagnoses and *GATA4* variation at the gene-level. Future studies should use this additional analysis to replicate these findings and identify any gene-level association between *GATA4* variants and alcohol-related clinical phenotypes. Confirming a gene-level association between *GATA4* and AUD would provide a target for identifying and treating maladaptive alcohol use.

Our finding of a relationship between SNP rs10112596 and an anxiety measure in the AA group with AUD is novel, as this marker has not yet been associated with any alcohol-related phenotype. ANP levels might underlie this correlation, as decreased ANP levels

Table 4. Associations between *GATA4* gene SNPs and Brief Scale for Anxiety (BSA) scores in AA sample with AUD

SNP	A1[a]	N	BETA	STAT	*P*-value	FDR
rs6990313	A	414	1.14	2.09	0.037	0.418
rs10105409	G	415	1.55	2.43	0.015	0.262
rs6601604	A	415	0.24	0.47	0.642	0.845
rs10112596	A	415	−2.24	−3.33	0.001*	0.032*
rs12550668	G	415	−0.76	−0.95	0.344	0.828
rs2898292	G	415	0.94	1.72	0.086	0.418
rs4840579	A	415	0.30	0.58	0.560	0.828
rs11250159	A	415	1.07	1.73	0.085	0.418
rs17153694	A	412	1.03	1.17	0.241	0.746
rs17153698	A	415	0.83	1.58	0.115	0.489
rs6983129	C	414	0.34	0.65	0.516	0.828
rs2898295	A	415	0.22	0.43	0.671	0.845
rs11250163	C	415	−0.27	−0.32	0.753	0.883
rs13275657	A	411	−1.33	−0.94	0.346	0.828
rs2029969	G	415	−0.38	−0.63	0.532	0.828
rs2173117	A	415	−0.44	−0.74	0.461	0.828
rs3779664	A	415	−0.44	−0.43	0.665	0.845
rs3735814	A	415	0.92	1.86	0.064	0.418
rs2740434	A	415	0.42	0.77	0.440	0.828
rs2645399	A	414	0.42	0.83	0.405	0.828
rs11784693	A	414	0.33	0.53	0.598	0.845
rs804283	G	415	0.06	0.08	0.940	0.940
rs17153747	G	415	−0.72	−0.61	0.545	0.828
rs804282	C	414	−0.04	−0.09	0.927	0.940
rs13264774	A	415	0.42	0.67	0.506	0.828
rs13273672	G	415	0.20	0.36	0.717	0.870
rs804280	C	415	0.46	0.87	0.384	0.828
rs3729856	G	415	0.17	0.10	0.924	0.940
rs867858	C	415	−0.10	−0.15	0.877	0.940
rs1062219	A	412	−1.15	−1.78	0.076	0.418
rs804290	A	415	−1.60	−1.40	0.161	0.548
rs11785481	A	415	0.33	0.22	0.825	0.935
rs12458	T	413	−0.32	−0.61	0.541	0.828
rs3203358	C	415	−1.57	−1.43	0.153	0.548

[a]Allele 1 refers to minor allele.

[b]Analysis controls for age, gender, African ancestry informative markers and European ancestry informative markers.

*$P < 0.05$.

have been associated with increased anxiety among individuals with AUD during detoxification (Kiefer *et al.*, 2002). This result also supports *GATA4* as a potential new target for research on comorbid AUD and anxiety, particularly among those of African ancestry. Consistent with a prior study that found a moderating effect of ethnicity on the association between alcohol abuse and an anxiety-related neuroendocrine biomarker, we found this association between *GATA4* and anxiety in only the AA group with AUD (Ransome *et al.* 2017). Our finding suggests that the minor allele in rs10112596 might provide a protective effect in lowering anxiety susceptibility among African Americans with AUD. However, given our sample size, this association should be further examined in future studies. We did not find any association that survived correction for multiple testing between *GATA4* SNPs and several other alcohol-related phenotypes. Further studies should utilize gene-level testing to determine whether there is a gene-level association between *GATA4* and comorbid AUD and alcohol-related phenotypes.

Our study endeavored to extend the knowledge of the genetic components relating to AUD. We did not replicate previous studies that found an association between *GATA4* variants and risk for AUD; however, further studies with larger samples and gene-level testing techniques are needed. We did find an association between rs10112596 and anxiety in the AA group, suggesting that this SNP may contribute to risk for AUD and anxiety in individuals of AA but not EA. This finding also implicates *GATA4* in the relationship between AUD and anxiety, indicating a possible protective effect of the rs10112596 A minor allele. This investigation contributes meaningfully to the field because it extends the discovery of certain genotypes that may be associated with a higher risk of developing and maintaining AUD, as well as those genotypes that constitute part of a genetic 'protective' factor. As medicine and treatment plans are becoming more personalized and patient-specific, it becomes crucial to elucidate the mechanisms behind the genetic contribution to complex disorders. Ideally, genetic information will provide us with the tools to better diagnose and prevent psychiatric disorders, including AUD. Genetics can also provide meaningful information regarding the underlying biological basis of a disease when designing treatment strategies. An understanding of genetic susceptibility to AUD may inform the development of individualized pharmacological interventions that may provide patient-specific drug efficacy.

SUPPLEMENTARY MATERIAL

Supplementary data are available at *Alcohol And Alcoholism* online.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

FUNDING

This work was supported by the National Institutes of Health (NIH) intramural funding [ZIA-AA000242; Section on Clinical Genomics and Experimental Therapeutics; to F.W.L.; Division of Intramural Clinical and Biological Research of the National Institute on Alcohol Abuse and Alcoholism (NIAAA)].

REFERENCES

American Psychiatric Association. (2000) *Diagnostic and Statistical Manual of Mental Disorders*, 4th edn, Text-revised. Washington, DC: American Psychiatric Association.

American Psychiatric Assocaition. (2013) *Diagnostic and Statistical Manual of Mental Disorders*, 5th edn. Washington, DC: American Psychiatric Association.

Amir O, Sagiv M, Eynon N, *et al.* (2010) The response of circulating brain natriuretic peptide to academic stress in college students. *Stress* **13**:83–90.

Barrett JC, Fry B, Maller J, *et al.* (2005) Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* **21**:263–5.

Becker HC, Mulholland PJ. (2014) Neurochemical mechanisms of alcohol withdrawal. *Handb Clin Neurol* **125**:133–56.

Benjamini Y, Hochberg Y. (1995) Controlling the false discovery rate—a practical and powerful approach to multiple testing. *J R Stat Soc* **57**:289–300.

Bouchery EE, Harwood HJ, Sacks JJ, *et al.* (2011) Economic costs of excessive alcohol consumption in the U.S., 2006. *Am J Prev Med* **41**:516–24.

Costa PT, McCrae RR. (2002) *NEO Personality Inventory-Revised (NEO-PI-R)*. Washington, DC: American Psychological Association.

Degenhardt F, Kramer L, Frank J, *et al.* (2016) Analysis of rare variants in the alcohol dependence candidate gene GATA4. *Alcohol Clin Exp Res* **40**:1627–32.

Delker E, Brown Q, Hasin DS. (2016) Alcohol consumption in demographic subpopulations: an epidemiologic overview. *Alcohol Res* **38**:7–15.

Edenberg HJ, Koller DL, Xuei X, *et al.* (2010) Genome-wide association study of alcohol dependence implicates a region on chromosome 11. *Alcohol Clin Exp Res* **34**:840–52.

First MB, Gibbon M, Spitzer RL, *et al.* (2002) *User's Guide for the Structured Clinical Interview for DSM-IV-TR Axis I Disorders—Research Version*. New York, NY: Biometrics Research Department, New York State Psychiatric Institute.

Glahn A, Rhein M, Heberlein A, *et al.* (2016) The epigenetic regulation of GATA4-dependent brain natriuretic peptide expression during alcohol withdrawal. *Neuropsychobiology* **74**:131–38.

Grant BF, Goldstein RB, Saha TD, *et al.* (2015) Epidemiology of DSM-5 alcohol use disorder results from the national epidemiologic survey on alcohol and related conditions III. *JAMA Psychiatry* **72**:757–66.

Hodgkinson CA, Yuan Q, Xu K, *et al.* (2008) Addictions biology: haplotype-based analysis for 130 candidate genes on a single array. *Alcohol Alcohol* **43**:505–15.

Jorde A, Bach P, Witt SH, *et al.* (2014) Genetic variation in the atrial natriuretic peptide transcription factor GATA4 modulates amygdala responsiveness in alcohol dependence. *Biol Psychiatry* **75**:790–7.

Karpyak VM, Winham SJ, Biernacka JM, *et al.* (2014) Association of GATA4 sequence variation with alcohol dependence. *Addict Biol* **19**:312–5.

Kenna GA, Roder-Hanna N, Leggio L, *et al.* (2012) Association of the 5-HTT gene-linked promoter region (5-HTTLPR) polymorphism with psychiatric disorders: review of psychopathology and pharmacotherapy. *Pharmgenomics Pers Med* **5**:19–35.

Kiefer F, Andersohn F, Jahn H, *et al.* (2002) Involvement of plasma atrial natriuretic peptide in protracted alcohol withdrawal. *Acta Psychiatr Scand* **105**:65–70.

Kiefer F, Witt SH, Frank J, *et al.* (2011) Involvement of the atrial natriuretic peptide transcription factor GATA4 in alcohol dependence, relapse risk and treatment response to acamprosate. *Pharmacogenomics J* **11**:368–74.

Malouff JM, Thorsteinsson EB, Rooke SE, *et al.* (2007) Alcohol involvement and the five-factor model of personality: a meta-analysis. *J Drug Educ* **37**:277–94.

McBride K, Nemer M. (2001) Regulation of the ANF and BNP promoters by GATA factors: lessons learned for cardiac transcription. *Can J Physiol Pharmacol* **79**:673–81.

Merikangas KR, Stevens D, Fenton B. (1996) Comorbidity of alcohol and anxiety disorders: the role of family studies. *Alcohol Health Res World* **20**:100–06.

Montgomery SA, Asberg M. (1979) A new depression scale designed to be sensitive to change. *Br J Psychiatry* **134**:382–9.

Poikolainen K. (2000) Risk factors for alcohol dependence: a case-control study. *Alcohol Alcohol* **35**:190–6.

Purcell S, Neale B, Todd-Brown K, *et al.* (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* **81**:559–75.

Ransome Y, Slopen N, Karlsson O, *et al.* (2017) The association between alcohol abuse and neuroendocrine system dysregulation: race differences in a National sample. *Brain Behav Immun* **66**:313–21.

Regier DA, Farmer ME, Rae DS, *et al.* (1990) Comorbidity of mental disorders with alcohol and other drug abuse. Results from the Epidemiologic Catchment Area (ECA) Study. *J Am Med Assoc* **264**: 2511–8.

Rietschel M, Treutlein J. (2013) The genetics of alcohol dependence. *Ann NY Acad Sci* **1282**:39–70.

Skinner HA, Allen BA. (1982) Alcohol dependence syndrome: measurement and validation. *J Abnorm Psychol* **91**:199–209.

Smith JP, Randall CL. (2012) Anxiety and alcohol use disorders: comorbidity and treatment considerations. *Alcohol Res* **34**:414–31.

Spielberger CD, Gorsuch RL, Lushene RE. (1970) *Manual for the State-Trait Anxiety Inventory*. Palo Alto, CA: Consulting Psychologists Press.

Stahre M, Roeber J, Kanny D, *et al.* (2014) Contribution of excessive alcohol consumption to deaths and years of potential life lost in the United States. *Prev Chronic Dis* **11**:E109.

Sullivan JT, Sykora K, Schneiderman J, *et al.* (1989) Assessment of alcohol withdrawal: the revised clinical institute withdrawal assessment for alcohol scale (CIWA-Ar). *Br J Addict* **84**:1353–7.

Tawa EA, Hall SD, Lohoff FW. (2016) Overview of the genetics of alcohol use disorder. *Alcohol Alcohol* **51**:507–14.

Treutlein J, Cichon S, Ridinger M, *et al.* (2009) Genome-wide association study of alcohol dependence. *Arch Gen Psychiatry* **66**:773–84.

Tyrer P, Owen RT, Cicchetti DV. (1984) The brief scale for anxiety: a subdivision of the comprehensive psychopathological rating scale. *J Neurol Neurosurg Psychiatry* **47**:970–5.

Zois E, Vollstadt-Klein S, Hoffmann S, *et al.* (2016) GATA4 variant interaction with brain limbic structure and relapse risk: a voxel-based morphometry study. *Eur Neuropsychopharmacol* **26**:1431–7.