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Association of *GATA4* sequence variation with alcohol dependence

Victor M. Karpyak, MD, PhD^{1,*,#}, Stacey J. Winham, PhD^{2,#}, Joanna M. Biernacka, PhD^{1,2}, Julie M. Cunningham, PhD³, Kriste A. Lewis, MS¹, Jennifer R. Geske, MS², Colin L. Colby, BA², Osama A. Abulseoud, MD¹, Daniel K. Hall-Flavin, MD¹, Larissa L. Loukianova, MD, PhD¹, Terry D. Schneekloth, MD¹, Mark A. Frye, MD¹, John A. Heit, MD⁴, and David A. Mrazek, MD¹

¹Psychiatry and Psychology, Mayo Clinic, Rochester, MN

²Health Sciences Research, Mayo Clinic, Rochester, MN

³Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN

⁴Division of Cardiovascular Diseases, Mayo Clinic, Rochester, MN

Abstract

To further explore reports of association of alcohol dependence and response to acamprosate treatment with the *GATA4* rs13273672 single nucleotide polymorphism (SNP), we genotyped this and 10 other *GATA4* SNPs in 816 alcohol dependent cases and 1248 controls. We tested for association of alcohol dependence with the 11 SNPs individually and performed a global test for association using a principle components analysis (PCA). Our analyses demonstrate significant association between *GATA4* and alcohol dependence at the gene-level ($p=0.009$) but no association with rs13273672. Further studies are needed to identify potential causal *GATA4* variation(s) and the functional mechanism(s) contributing to this association.

Keywords

GATA4; Alcohol dependence; Genetic association; Gene level test

The *GATA4* gene encodes GATA-motif binding protein type 4 (GATA4) – a transcription factor controlling expression of multiple proteins including drug metabolizing cytochrome P450 2C9 (CYP2C9) and cytochrome P450 2C19 (CYP2C19) enzymes, human brain natriuretic peptide (hBNP) and atrial natriuretic peptide (ANP) (He, Mendez and LaPointe, 2002; Mwinyi et al., 2010). Recent reports found association between the *GATA4* rs13273672 G allele and decreased variance in ANP expression with an increased propensity to relapse in alcohol dependent subjects treated with acamprosate (Kiefer et al., 2011). Rs13273672 was associated with alcohol dependence in two out of four GWAS completed so far (Edenberg et al., 2010; Treutlein et al., 2009) (Bierut et al., 2010; Zuo et al., 2012)

*Corresponding author: Victor M. Karpyak, M.D., Ph.D., Department of Psychiatry and Psychology, Mayo Clinic Rochester 200 First Street SW, Rochester, MN 55905, Phone: 507-255-9391, Fax: 507-255-9416 karpyak.victor@mayo.edu.

#These two authors contributed equally to this work.

AUTHORS CONTRIBUTION

VMK and JMB were responsible for the study concept and design. SJW and JMB designed the analysis plan and oversaw the statistical analysis of the data, while JRG and CLC managed the data, performed the analyses and prepared the results summaries. JMC oversaw the genotyping and quality control. VMK and SJW drafted the manuscript. VMK, DKH-F, LLL and TDS evaluated study subjects. JAH designed and directed study which provided control subjects. VMK, SJW, JMB, JRG, CLC, JMC, KAL, OAA, DKH-F, LLL, TDS, MAF, JAH and DAM critically reviewed and approved the final version of the manuscript.

and model studies demonstrate that high doses of ethanol augment the expression of *GATA4* (Zhong et al., 2010).

Yet the physiological mechanisms underlying potential association of alcohol dependence with sequence variation in *GATA4* gene and expression of the *GATA4* protein remain unknown. Moreover, the association of alcohol dependence with variation in *GATA4* gene has not been comprehensively investigated and it remains unknown whether rs13273672 has a functional role or is in linkage disequilibrium (LD) with other functional SNP(s). Therefore, investigation of the association between alcohol dependence and other variations in the *GATA4* gene is necessary. In this study we investigated the association between alcohol dependence and 11 SNPs within *GATA4*, including rs13273672, and evaluated the association of alcohol dependence with total variation in the *GATA4* gene.

This study was approved by the Institutional Review Board of Mayo Clinic Rochester. All subjects provided informed consent and permission to use collected information in studies of alcohol dependence and related phenotypes. Alcohol dependent subjects (n=936) were diagnosed according to DSM-IV-TR criteria and participated in ongoing or completed studies focused on clinical and genetic predictors of alcohol dependence and related phenotypes (Karpyak et al., 2010; Karpyak et al., 2009; Schneekloth et al., accepted for publication 2012). Control subjects (n=1302) were selected from a group of controls that previously participated in a GWAS of venous thrombosis carried out at Mayo Clinic and gave consent for general research use (Heit et al., 2011). The case subjects were younger (49.15 $\pm$ 12.13 vs. 57.22 $\pm$ 15.93 years, $p < 0.0001$) than the control subjects and included more males (67.9% vs. 48.3%, $p < 0.0001$).

For cost savings and efficiency, previously genotyped controls were used for this study, and thus genotyping was performed separately in cases and controls. As part of a GWAS of venous thrombosis (Heit et al., 2011), 1302 controls were previously genotyped with the Illumina 660 genome-wide SNP array, which contained eleven SNPs within the *GATA4* gene (Table 1), including rs13273672. The LD pattern of these SNPs is shown in Supporting Figure 1. To investigate the association with alcohol dependence, these 11 *GATA4* SNPs were subsequently genotyped in 936 alcohol dependent cases using the Illumina BeadXpress platform with Illumina GoldenGate SNP array. Forty-three ancestry informative markers (Supporting Table 1) were also genotyped to allow for verification of self-reported ancestry.

As part of case genotyping quality control, 4 duplicate samples were included with 100% concordance. One CEPH parent-child trio was genotyped six times with 100% concordance and no Mendelian errors. Furthermore, one of the CEPH controls genotyped with the cases was also genotyped with the control samples. The genotypes from the two platforms were 100% concordant. Twenty-one case samples failed genotyping. All SNPs had call rates greater than 97% (mean=99.9%). All SNPs had minor allele frequencies greater than 0.10 and none exhibited departures from Hardy-Weinberg Equilibrium (all $p > 0.01$).

Thirteen potential control subjects had a documented history of alcohol dependence in the medical records and were excluded from analyses. To avoid the confounding effects of population stratification, only subjects with self-reported “white, non-Hispanic” race were included for analyses, and STRUCTURE (Pritchard, Stephens and Donnelly, 2000) analysis of 43 ancestry informative markers was used to verify genetic ancestry. One case and one control subject were excluded because they were estimated to have greater than 30% African ancestry, leading to a final sample of 816 cases and 1248 controls.

Associations between the 11 *GATA4* SNPs and alcohol dependence were assessed using single SNP and gene-level tests of association. For each SNP, the association with alcohol

dependence was evaluated using univariate logistic regression, with SNP genotypes coded as 0, 1 or 2 - the number of copies of the minor allele. Statistical significance was determined with a likelihood ratio test and permutation testing (10,000 permutations) was used for multiple testing correction.

In addition to tests of single SNP association, we performed global tests for association of alcohol dependence with all measured variation in the *GATA4* gene using a principle components analysis (PCA) approach (Gauderman et al., 2007). Data from all 11 SNPs were transformed into 11 principle components (linear combinations of the SNPs) that explain the total SNP variance. We retained the first four components (which together explained more than 80% of the SNP variation) as predictors in a logistic regression model, and used a four degree-of-freedom likelihood ratio test to assess the joint effect of the principle components on alcohol dependence. For further verification of these results, we also performed a gene-level test using a joint SNP approach. We included all 11 SNPs simultaneously in a logistic regression model and tested for association using an 11 degree-of-freedom likelihood ratio test. As only one gene was evaluated for association with alcohol dependence, correction for multiple testing is not necessary for the gene-level tests. All statistical analyses were performed in R Statistical Software, version 2.14.0.

Although none of the SNP-specific results were significant after correction for multiple testing, 5 of the 11 *GATA4* SNPs were nominally associated with alcohol dependence at the 0.05 significance level (Table 1). The minor allele of rs809204 was estimated to be a risk variant, whereas the minor alleles of rs10112596, rs804283, rs6601604, and rs12550668 were estimated to have protective effects. We did not observe significant evidence for association of alcohol dependence with candidate SNP rs13273672 ($p=0.59$). However, the PCA approach provided significant evidence of association between *GATA4* and alcohol dependence at the gene level ($p=0.009$), which was also verified by joint-SNP gene-level analysis ($p=0.037$). Both gene-level approaches revealed statistically significant evidence of association between alcohol dependence and variation in the *GATA4* gene.

The *GATA4* rs13273672 SNP was among association findings in two GWAS of alcohol dependence (at the level of significance below 0.05) (Edenberg et al., 2010; Treutlein et al., 2009), where the estimated odds ratio was 1.20 and 1.19, respectively. However, we did not find this SNP to be associated with alcohol dependence and the estimated odds ratio in our study ($OR=1.04$) was consistent with the results reported in two other GWAS (Bierut et al., 2010; Zuo et al., 2012). None of the other *GATA4* SNPs considered here have been previously reported to be significantly associated with alcohol dependence in GWAS. We also did not detect association with any of the individual *GATA4* SNPs after correction for multiple testing. However, our findings provide evidence of association between alcohol dependence and variation in *GATA4* at the gene level. It is possible that *GATA4* sequence variation(s) other than those tested in this study are responsible for the observed association. Further investigation is necessary to identify the functional variation that may play a role in the observed association and study the underlying mechanism driving the association.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Association between alcohol dependence and *GATA4* SNPs

SNP	BP position (Chromosome 8)	Major/ Minor Allele	MAF in cases	MAF in controls	OR (95% CI)	P-value uncorrected	P-value corrected
rs13273672	11649790	A/G	0.332	0.325	1.04 (0.91, 1.18)	0.59	1.00
rs6601604	11612927	G/A	0.278	0.307	0.87 (0.76, 1.00)	0.044	0.30
rs10112596	11617211	G/A	0.151	0.179	0.82 (0.69, 0.97)	0.018	0.14
rs12550668	11617363	G/A	0.363	0.393	0.88 (0.77, 1.00)	0.049	0.32
rs1390950	11633238	A/G	0.374	0.366	1.03 (0.91, 1.18)	0.61	1.00
rs3735814	11644805	A/G	0.450	0.480	0.93 (0.82, 1.06)	0.28	0.91
rs7006733	11645399	A/G	0.325	0.350	0.90 (0.79, 1.03)	0.113	0.60
rs804283	11648452	A/G	0.257	0.286	0.87 (0.75, 1.00)	0.044	0.30
rs17153747	11648762	A/G	0.123	0.123	1.00 (0.82, 1.21)	0.962	1.00
rs867858	11653747	A/G	0.302	0.317	0.93 (0.82, 1.07)	0.317	0.94
rs809204	11656572	A/C	0.388	0.353	1.15 (1.02, 1.31)	0.027	0.20

MAF, minor allele frequency; OR, odds ratio.

Nominally significant p-values (at $p < 0.05$ level) presented in bold.