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Genome-wide Association Studies of Maximum Number of Drinks

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Abstract

Maximum number of drinks (MaxDrinks) defined as “Maximum number of alcoholic drinks consumed in a 24-hour period” is an intermediate phenotype that is closely related to alcohol dependence (AD). Family, twin and adoption studies have shown that the heritability of MaxDrinks is approximately 0.5. We conducted the first genome-wide association (GWA) study and meta-analysis of MaxDrinks as a continuous phenotype. 1059 individuals were from the Collaborative Study on the Genetics of Alcoholism (COGA) sample and 1628 individuals were from the Study of Addiction – Genetics and Environment (SAGE) sample. Family sample with 3137 individuals was from the Australian twin-family study of alcohol use disorder (OZALC). Two population-based Caucasian samples (COGA and SAGE) with 1 million single-nucleotide polymorphisms (SNPs) were used for gene discovery and one family-based Caucasian sample was used for replication. Through meta-analysis we identified 162 SNPs associated with MaxDrinks ($p < 10^{-4}$). The most significant association with MaxDrinks was observed with SNP rs11128951 ($p = 4.27 \times 10^{-8}$) near SGOL1 gene at 3p24.3. Furthermore, several SNPs (rs17144687 near DTWD2, rs12108602 near NDST4, and rs2128158 in KCNB2) showed significant associations with MaxDrinks ($p < 5 \times 10^{-7}$) in the meta-analysis. Especially, 8 SNPs in DDC gene showed significant associations with MaxDrinks ($p < 5 \times 10^{-7}$) in the SAGE sample. Several flanking SNPs in above genes/regions were confirmed in the OZALC family sample. In conclusions, we identified several genes/regions associated with MaxDrinks. These findings can improve the understanding about the pathogenesis of alcohol consumption phenotypes and alcohol-related disorders.

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Keywords

Maximum number of drinks; Genome-wide association; Meta-analysis; SGOL1; DTWD2; NDST4; KCNB2; DDC

1. Introduction

Maximum number of drinks (MaxDrinks) defined as “Maximum number of drinks consumed in a 24-hour period” is an alcoholism-related phenotype that could be a surrogate to alcohol dependence (AD) and a quantitative measure to grade non-alcoholic individuals (Bierut et al., 2002). There have been an increasing number of reports on binges, alcohol-related life problems such as physiological complications, alcohol-related emotional/psychiatric symptoms in the groups with larger maximum number of drinks (Schuckit et al., 1998). Family, twin and adoption studies have shown that the heritability of MaxDrinks (Saccone et al., 2000) is approximately 0.5. Studies of the genetic basis of MaxDrinks can provide more information for the understanding of AD, and enhance the development of efficient prevention strategies and personalized treatments. Saccone et al. (2000) conducted genome-wide linkage analysis of MaxDrinks and detected linkage region of the alcohol dehydrogenase gene cluster on chromosome 4 (LOD = 3.5). Furthermore, Saccone et al. (2005) detected linkage to chromosomes 2 and 7 using a two-stage method. De Andrade et al. (2005) identified suggestive linkage on chromosomes 1, 4, 10 and 13. Linkage for MaxDrinks was also detected on chromosomes 7 (Chen et al., 2005; Saccone et al., 2005). In an Irish affected sib pair study, Kuo et al. (2006) reported that MaxDrinks was associated with regions on chromosomes 12 and 18.

The genome-wide association (GWA) study has been successfully used as an important tool for identifying regions of human genome that are associated with more than 40 different common diseases. This approach has provided new insights into pathophysiology and suggested previously unsuspected etiologic pathways for common diseases that could be of use in identifying new therapeutic targets and in developing targeted interventions based on genetically defined risk (Manolio et al., 2008). Recently, there are several completed GWA studies for alcohol dependence (Bierut et al., 2010; Edenberg et al., 2010; Lind et al., 2010; Treutlein et al., 2011). However, based on our knowledge, no GWA study has been conducted on MaxDrinks as a quantitative phenotype in the literature. In this study, we performed a meta-analysis using two genome-wide data to detect genetic variants that may influence MaxDrinks in both Caucasian samples. We also used the dataset from The Australian twin-family study of alcohol use disorder (OZALC study) for replication.

2. Materials and methods

2.1. Study samples

2.1.1. The COGA sample—The Collaborative Study on the Genetics of Alcoholism (COGA) Case Control Study is a case-control GWA study of AD. It contains about 1 million Illumina SNPs (1,069,796 SNPs), and 1234 cases with AD and 711 controls (Edenberg et al., 2010). Phenotypes include AD as a binary trait according to DSM-IV diagnosis. Besides, another quantitative and heritable phenotype MaxDrinks that measured

the maximum number of drinks a person has consumed in a 24-hour period has been included in the study which increased the study power (Edenberg et al. 2002). In the present study, we chose 1059 Caucasian (non-Hispanic) individuals (572 males and 487 females) with MaxDrinks.

2.1.2. The SAGE sample—The Study of Addiction – Genetics and Environment (SAGE) is a comprehensive GWA study using approximately 4,000 unrelated subjects of European and African-American descent. Cases with AD include 1,944 subjects with the primary phenotype having been DSM-IV AD (Bierut et al., 2010). Controls consist of 1,965 subjects who have used alcohol, but have never been addicted to alcohol or other illicit substances. In order to screen for substance dependence, MaxDrinks were asked to potential control subjects. In the present study, we used 1628 Caucasian individuals with MaxDrinks (629 males and 999 females) from the combined data of the Family Study of Cocaine Dependence (FSCD), and the Collaborative Genetic Study of Nicotine Dependence (COGEND) in the SAGE study. It contains about 1 million Illumina SNPs (1,069,796 SNPs).

2.1.3. The OZALC sample—The Australian twin-family study of alcohol use disorder (OZALC study) derives from telephone diagnostic interview studies of two general population volunteer cohorts of Australian twins (cohort 1, mostly born 1940–1964; cohort 2, born 1964–71) and the spouses of the former cohort – a total of over 11,000 families. The data used in the present study is from the publicly available data from the Genome wide Association Study of Alcohol Use and Alcohol Use Disorder in Australian Twin-Families (OZALC GWAS) – Study Accession: phs000181.v1.p1. The details about these subjects were described elsewhere (Grant et al., 2009; Lind et al., 2010). Genotyping data using the ILLUMINA HumanCNV370v1 (total 343,955 SNPs) are available for 4,119 individuals in this data set. After merging with pedigree and phenotypes, we removed one from each of 44 MZ twins and 72 outliers based on the data description, and 669 individuals with unknown case status. Consequently, there were 3137 individuals with MaxDrinks left for further analysis (1708 males and 1429 females).

The basic characteristics of the subjects in these 3 samples are presented in Table 1.

2.2. Statistical analyses

2.2.1. Genome-wide association analysis—For the initial GWA analysis, HelixTree Software (http://www.goldenhelix.com/SNP_Variation/HelixTree/index.html) was used to assess control genotype data for conformity with Hardy-Weinberg equilibrium (HWE). To test for association with MaxDrinks as a quantitative trait, linear regression, adjusted for age and sex, was performed by PLINK 1.07 (Purcell et al., 2007) to obtain the regression coefficient and standard error as well as Wald test asymptotic p-value. For statistical significance, we used a significance level of $\alpha = 5 \times 10^{-7}$ (Wellcome Trust Case Control Consortium, 2007). At the same time, we also used a less stringent criterion of “suggestive association” with a cut-off of $\alpha = 10^{-4}$. In addition to obtaining nominal p-values, empirical p-values were generated by 100,000 permutation tests using the Max (T) permutation procedure implemented in PLINK. In this procedure, two sets of empirical significance

values were calculated: pointwise estimates of an individual SNP's significance (empirical pointwise p-values) and corrected values for multiple testing (corrected empirical p-values).

2.2.2. Meta-analysis—The COGA and SAGE samples used the same genotyping platform: Illumina Human 1M BeadChips (both datasets have 1,069,796 SNPs). Results from the two GWA analyses were meta-analyzed by combining the separate results of COGA and SAGE samples (regression coefficient and standard error) into one meta-analysis of overall effects. For meta-analysis of two datasets, the basic meta-analysis function in PLINK was applied. The between-study heterogeneity was tested by the χ^2 -based Cochran's Q statistic. Fixed-effect model was used due to the homogeneity Q statistic and its associated p-value was reported to provide support for this decision. Fixed-effect meta-analysis p-values and fixed-effect ORs were estimated.

2.2.3. Family-based association analysis in the OZALC sample—In this study, family-based association analysis was performed in the OZALC sample by using the PBAT v3.61 (Hoffmann and Lange, 2006), which can handle nuclear families, as well as extended pedigrees. For family-based association analysis, the additive model was applied. To deal with the multiple testing in the family-based association study, we used software QVALUE (<http://genomics.princeton.edu/storeylab/qvalue/>) to calculate the false discovery rate (FDR) (Storey, 2002).

2.2.4. Cis-acting expression of quantitative locus (Cis-eQTL) analysis on the risk SNPs in two primary human cells—To examine whether the risk SNPs (listed in 1st column in Tables 2, 3 and 4) influenced the transcript-level expression changes of local genes (listed in 4th column in Tables 2, 3 and 4), we also tested the associations between the genotypes of these risk SNPs and the expression levels of these genes in two European samples. Expression data in 93 autopsy-collected frontal cortical brain tissue samples with no defined neuropsychiatric condition and 80 peripheral blood mononuclear cell (PBMC) samples collected from living healthy donors were evaluated (Heinzen et al., 2008). Each of these associations was analyzed using a linear regression model by correcting for age, sex, source of tissues, and principle component scores.

3. Results

3.1. Genotype quality control

We removed SNPs with HWE $p < 0.0001$ (in controls) or call rates $< 95\%$ or minor allele frequency (MAF) $< 1\%$ leaving 778,974 SNPs for the COGA sample and 818,773 SNPs for the SAGE sample for further analysis. P-values from the Cochran's Q values were provided for evaluating and testing the heterogeneity of variance in the effect sizes of selected studies.

3.2. Genome-wide association analysis

Through meta-analysis we have identified 162 SNPs associated with MaxDrinks with $p < 10^{-4}$, of which 81 SNPs are located in known genes (supplementary Table S1). In Table 2, we listed the top 25 findings ranked by their p-values. We focused on the regions around the

first 6 SNPs with $p < 10^{-6}$. Two SNPs reached conservative per-test significance level ($p < 5 \times 10^{-7}$) (26): The strongest associated marker was rs11128951 ($p = 4.27 \times 10^{-8}$, 2.19×10^{-3} , 7.47×10^{-7} , for meta-analysis, SAGE and COGA samples, respectively) located at 3p24.3 near SGOL1 gene while the second best novel hit was rs17144687 ($p = 9.27 \times 10^{-8}$, 1.06×10^{-6} , 2.51×10^{-2} , for meta-analysis, SAGE and COGA samples, respectively) at 5q23.1 near DTWD2 gene. Furthermore, the third and fourth significant associations were observed with rs12108602 near NDST4, and rs2128158 in KCNB2 ($p < 5 \times 10^{-7}$). In addition, significant results of our top findings were consistent for COGA and SAGE sample separately ($p\text{-sa} < 0.05$ and $p\text{-co} < 0.05$). Applying a permutation procedure for multiple test correction also yielded significant p-values (corrected empirical p-values). 19 of the top 25 SNPs for the SAGE sample had corrected $p < 0.05$ while 10 of the top 25 SNPs for the COGA sample had corrected $p < 0.05$ (Table 2). Among these 25 top risk SNPs, we found two SNPs, i.e., rs1041264 and rs17148121 at RAPGEF1, that had significant *cis*-acting regulatory effects on the transcript expression of RAPGEF1 in the brain ($p = 0.029$ and 0.018 , respectively) (Table 5).

Interestingly, we noticed that nine SNPs around 7p12.1–7p12.2 in the SAGE sample reached genome-wide significance level ($p < 5 \times 10^{-7}$), whereas all of them were not significant in the COGA sample (Table 3). Especially, 8 SNPs in DDC gene showed significant associations with MaxDrinks ($p < 5 \times 10^{-7}$) in the SAGE sample (Table 3). All the nine p-values based on Q statistic were significant, which indicated that there was heterogeneity for these 9 SNPs between COGA and SAGE samples (Table 3). In addition, we found two SNPs, i.e., rs11575522 and rs11575542 (Arg462Gln) at DDC, that had significant *cis*-acting regulatory effects on the transcript expression of DDC both in the brain and PBMC ($p < 0.05$) (Table 5).

3.3. Family-based association analyses in the OZALC sample

For the top identified 6 genes/loci (rs11128951 near SGOL1, rs17144687 near DTWD2, rs12108602 near NDST4, KCNB2, rs2677485 near NR4A2 and DDC) by the meta-analysis and GWA analysis, we selected 282 SNPs from the OZALC dataset to perform a replication analysis of our results. For each SNP in meta-analysis which is not within a known gene, we chose 20 flanking SNPs in the OZALC dataset while we included all the markers in each of the genes (KCNB2, DDC and GRB10) from the OZALC sample. Based on the QVALUE, when the p -value cutoff is 0.05, 20 out of 282 SNPs were associated with MaxDrinks in the OZALC sample, with FDR of 0.54 (Table 4). Three genes (NDST4, KCNB2 and DTWD2) were confirmed to be associated with MaxDrinks in the replication study by testing the flanking SNPs in the OZALC family sample ($p < 0.05$). In Table 4, twelve flanking SNPs that located near NR4A2 at 2q24.1 have been replicated in the family sample while the top hit was rs1402071 ($p = 0.0019$). In addition, one SNP rs7624305 near SGOL1 and one SNP rs4947510 within DDC gene revealed borderline significant association with AD in the family sample ($p = 0.106$ and 0.0916 , respectively) (supplementary Table S2).

3.4. Cis-acting expression of quantitative locus (Cis-eQTL) analysis on the risk SNPs in two primary human cells

rs12471739 at NR4A2 had significant *cis*-acting regulatory effects on the transcript expression of NR4A2 in the PBMC ($p < 0.048$) (Table 5). For KCNB2, one SNP rs2929576

with $p = 2.71 \times 10^{-7}$ in the meta-analysis was confirmed in the family sample ($p = 0.0142$). Furthermore, five flanking SNPs were located in KCNB2 on chromosome 8 (Table 4). rs2958414 at KCNB2 had significant *cis*-acting regulatory effects on the transcript expression of KCNB2 in the brain ($p < 0.018$) (Table 5). In addition, several flanking SNPs demonstrated borderline associations with MaxDrinks that have been listed in the supplement Table S1. For example, rs7624305 ($p = 1.06 \times 10^{-1}$) near SGOL1, rs2620418 ($p = 1.03 \times 10^{-2}$) and rs11938588 ($p = 2.03 \times 10^{-2}$) in NDST4, rs2620418 ($p = 2.09 \times 10^{-2}$) in DTWD2, rs7624305 ($p = 1.06 \times 10^{-1}$) in 3p24.3 and rs4947510 ($p = 9.16 \times 10^{-2}$) in DDC. However, the top hit SNP from meta-analysis (rs11128951) was not confirmed in the family sample possibly due to cause of the ambidirectional effect indicated by the significant Cochran's Q value ($p = 0.04$). The *cis*-eQTL signal of this top hit SNP was not detected either. Besides, all other signals have the same direction of effect ($p = 0.05$).

4. Discussion

To our knowledge, this is the first GWA study and meta-analysis of MaxDrinks as a quantitative phenotype. Based on meta-analysis of two GWA data of Caucasian samples, we have identified a number of novel genes/regions associated with MaxDrinks. Six genes/regions (SGOL1, DTWD2, NDST4, KCNB2, NR4A2 and DDC), most of which had significant *cis*-eQTL signals, are most promising. In the replication study by using a family sample, several flanking SNPs in those six genes/regions were confirmed.

SNP rs11128951 near SGOL1 (Also known as SGO, SGO1) at 3p24.3 reached the genome-wide significant level ($p < 5 \times 10^{-7}$). Salic et al. (2004) concluded that SGOL1 is required for mitotic progression and chromosome segregation and provides a link between sister centromere cohesion and microtubule interactions at kinetochores. Riedel et al. (2006) showed that SGOL1 recruited to centromeres a specific form of protein PP2A in fission and budding yeast and concluded that efficient cleavage of Rec8 required phosphorylation of cohesion and that this was blocked by PP2A at meiosis I centromeres. SGOL1, which encodes a centromeric protein that belongs to the shugoshin family, is essential in chromosome cohesion during mitosis. It prevents premature dissociation of cohesion complex from centromeres after prophase, when most of cohesion complex dissociates from chromosomes arms (Kitajima et al., 2006). Experiment has confirmed that centrosome/spindle pole sSgo1 signals, the shorter isoform, are detected in interphase and mitotic cells while sSGO1 plays an essential role in protecting centriole cohesion (Wang et al. 2008). In 2008, Yamagishi et al. (2008) demonstrated that the recruitment of SGOL was the important primary role for centromeric heterochromatin in ensuring eukaryotic chromosome segregation. Uncharacterized interphase functions of SGOL1 at the centromeres may be regulated by interaction between heterochromatin protein 1 (HP1) and SGOL1 (Kang et al., 2011). Till now, no association between SGOL1 gene and any psychiatric disorders and related phenotypes has been found. SGOL1 has also been identified to be strongly overexpressed in breast cancer and had higher splice indices in malignant tumors than in benign tissues (Andre et al., 2009). Recently, Kahyo et al. (2011) reported that chromosome instability was more likely in SGOL1-downregulated colorectal cancer and there was an association between SGOL1 variant and colon cancer. It has been reported that alcohol consumption is an important cause of cancer worldwide (Boffetta and Hashibe, 2006;

Druesne-Pecollo et al., 2009). For example, excessive alcohol intake is one of the important risk factors for colorectal cancer (Orbell and West, 2010). In addition, the functional variants in genes involved in alcohol metabolism might result in differences between individuals in exposure to carcinogenic acetaldehyde, suggesting a possible interaction of genetic susceptibility and alcohol exposure in cancer (Druesne-Pecollo et al. 2009).

The DTWD2 gene at 5q23.1 was identified through SNP rs17144687 in the meta-analysis. This locus also reached genome-wide significant associations ($p < 5 \times 10^{-7}$) with several flanking SNPs meeting the borderline significant associations in the replication study (rs6868097 with $p = 0.0209$ and rs6874109 with $p = 0.0695$). This gene may be functional because rs6868097 has significant *cis*-acting regulatory effect on DTWD2 transcript expression. Unfortunately, there is little other known biological function of DTWD2 that has been reported up to now. A rare copy number variation was identified in DTWD2 in age-related disorders of blindness, with a focus on primary open-angle glaucoma (Davis et al., 2011). Interestingly, one study showed a sudden blindness in a patient with alcohol abuse (Oksanen, 2002). It possibly implies that DTWD2 may play a role in disorders of blindness and alcohol consumption. Recently, several polymorphisms in DTWD2 were reported to have moderate associations with celiac disease with p -values between 0.000275 and 0.004 (Trynka et al., 2009).

The third locus was rs12108602 closed to NDST4 at 4q26 which showed significant results in both COGA ($p=4.32 \times 10^{-4}$) and SAGE ($p=6.24 \times 10^{-5}$) samples. In addition, two of the SNPs have been replicated in the OZALC sample (rs2620418 with $p = 0.0103$ and rs11938588 with $p = 0.0203$). rs11938588 has significant *cis*-acting regulatory effect on NDST4 transcript expression. NDST4 whose alias as N-deacetylase/N-sulfotransferase (heparan glucosaminyl) 4 has weak deacetylase activity but high sulfotransferase. It has clear differences in distribution and enzymatic properties. Different combinations of the isozymes may account for some of the differences seen in heparan sulfates from various tissues (Aikawa et al., 2001). Previous genome linkage study identified chromosome 4 in the vicinity of the alcohol dehydrogenase (ADH) gene cluster (Saccone et al., 2000) while additional analyses of chromosome 4 showed modest evidence for both linkage and association. Recently, SNP rs7671475 in NDST4 showed a borderline association with AD (Kalsi et al., 2010).

Next interesting locus was KCNB2 at 8q13.2. Two SNPs (rs2128158 and rs2929567) had significant associations with Maxdrinks in COGA and SAGE samples. Noticeably, rs2929567 had been well replicated in the OZALC sample ($p=0.0142$) while 4 additional SNPs in KCNB2 also showed borderline significant associations with Maxdrinks. KCNB2 may be functional because *cis*-eQTL signal was detected in this gene in this study. KCNB2 has been reported to be highly associated with left ventricular diastolic dimension, which is a heritable trait that is associated with cardiovascular disease (Vasan et al., 2007). Furthermore, KCNB2 interacted with CACNB2 in influencing common migraine (Nyholt et al., 2008). In a genome wide expression analysis, KCNB2 was found to be associated with long-term changes after acute nicotine exposure that may have complicated influences related to the function of the nervous system (Wang et al., 2011).

Another locus was rs2677485 near NR4A2 (also known as NOT; RNR1; HZF-3; NURR1; TINUR) at 2q24.1. Although it was at the marginal significant association level in the meta-analysis ($p=5.5\times 10^{-7}$), two other SNPs (rs4407211 with $p=3.24\times 10^{-6}$ and rs13035632 with $p=6.11\times 10^{-6}$) were also reported in the top identified SNPs. In addition, NR4A2 had the most flanking SNPs that were replicated in the OZALC sample and a total of 12 loci were confirmed in the replication study (Table 4). *Cis*-eQTL signal was also detected in this gene. NR4A2 was an orphan nuclear receptor transcripts factor that appeared to be predominantly brain-specific and expressed in dopaminergic neurons (Zetterstrom et al., 1996). Interestingly, Werme et al. (Werme et al., 2003) reported the involvement of NR4A2 in the transition to a state of high ethanol consumption as well as in the development of a high amount of wheel running in mice. Findings of NR4A2 variation have been reported to be associated with nicotine and AD (Bergen et al., 2009; Ishiguro et al., 2002).

Interestingly, 9 SNPs at 7p12.2 (8 SNPs in DDC gene and 1 SNP in GRB10 gene) reached genome-wide significance level ($P<5\times 10^{-7}$) in the SAGE sample (Table 4). Two of them, including a nonsynonymous SNP, had replicable *cis*-acting regulatory effects on the DDC transcript expression, which increased the possibility that DDC might play a functional role in risk for disease. DOPA decarboxylase (DDC; also known as L-amino acid decarboxylase; AADC) is involved in the synthesis of dopamine, norepinephrine and serotonin. DDC encodes a protein that is responsible for catalyzing the decarboxylation of L-3,4-dihydroxyphenylalanine (DOPA) to dopamine, L-5-hydroxytryptophan to serotonin and L-tryptophan to tryptamine (Hopfer et al., 2001). Chromosome 7 has been previously reported to have linkage with MaxDrinks (Saccone et al., 2005) by using the COGA sample. DDC is a potential gene that has association with nicotine dependence and smoking behavior (Yu et al., 2006). Agrawal et al. (2011) reported that multiple markers in DDC were associated with alcohol consumption and suggested the potential role of the serotonin synthesis pathway in alcohol consumption in a sample of 827 young women. DDC gene which involved in the synthesis of dopamine has been associated with increased motivation to consume alcohol in regular smokers (Kristjansson et al., 2012).

Comparing with other studies, our findings have some similarities and dissimilarities. First, this is the first GWA meta-analysis of MaxDrinks as a continuous phenotype. Meta-analysis would provide more power to identify SNP associated with MaxDrinks. Our results provide support for the findings of several genetic linkage analyses for alcohol consumption or AD. In our study, we used a meta-analysis and GWA on two population samples with approximately 1 million SNPs each and discovered associations of SNPs with significant *p*-values on chromosomes 3p24.3, 5q23.1, 4q26, 8q13.2, 2q24.1 and 7p12.2, which covered most of the reported chromosomes from the previous linkage analysis of MaxDrinks (Saccone et al., 2000; Saccone et al., 2005). Second, we performed a family-based analysis of the OZALC family dataset for replication and successfully replicated a number of SNPs. Third, through GWA studies and meta-analysis we found six genes/regions (SGOL1, DTWD2, NDST4, KCNB2, NR4A2 and DDC) significantly associated with MaxDrinks. Furthermore, these genes/regions were partially confirmed in a family-based analysis of the OZALC sample. However, one limitation of this study is the family sample that we used for the replication has a relatively small sample size compared to the ones used in the meta-

analysis. Also, SNP panel from OZALC has limited coverage of the genome. Associations with these markers and gene regions require further replications in other study samples as well as more functional studies before any statement about causality is warranted.

Conclusions

We identified several MaxDrinks associated genetic variants. Specifically, six genes/regions (SGOL1, DTWD2, NDST4, KCNB2, NR4A2 and DDC) showed significant results through GWA and meta-analysis. To our knowledge, this is the first meta-analysis that using GWA analysis of MaxDrinks as an alcohol-related phenotype. The results showed significant associations of SNPs at 3p24.3, 5q23.1, 4q26, 8q13.2, 2q24.1, and 7p12.2. The identification of specific genes influencing MaxDrinks will enable researchers to begin to decipher how the effects of these genes are modified by specific environmental influences. These findings may serve as a resource for replication in other populations and provide a foundation for future investigations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abuse and Alcoholism, and the NIH contract “High throughput genotyping for studying the genetic contributions to human disease” (HHSN268200782096C). The datasets used for the analyses described in this manuscript were obtained from dbGaP at http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000092.v1.p1 through dbGaP accession number phs000092.v1.p.1.

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Table 1

Descriptive characteristics of the subjects with MaxDrinks

Parameter	COGA	SAGE	OZALC
Number	1059	1628	3137
Women (%)	487(46%)	999(61%)	1429(46%)
Age (years)			
Mean $\pm$ SD	40.8 $\pm$ 11.4	35.7 $\pm$ 7.7	42.9 $\pm$ 8.2
MaxDrinks			
Mean $\pm$ SD	23.9 $\pm$ 21.1	17.5 $\pm$ 17.7	21.0 $\pm$ 14.9

Table 2

Top 25 SNPs associated with MaxDrinks in the meta-analysis Study

SNP	BAND	Position (BP)	GENE	AI	P-net	Q-meta	MAF-s	HWE-s	β-sa(SE)	EMP2-sa	P-sa	MAF-c	HWE-c	β-c(SE)	EMP2-co	P-co
rs11128951	3p24.3	20350550	near SGOL1	G	4.27E-08	0.04	0.19	0.72	2.31(0.75)	7.44E-01	2.19E-03	0.21	0.51	4.82(0.97)	2.00E-04	7.47E-07
rs17144687	5q23.1	118187834	near DTWD2	C	9.27E-08	0.48	0.02	0.32	9.53(1.95)	1.17E-03	1.06E-06	0.02	0.58	6.94(3.10)	4.18E-01	2.51E-02
rs12108602*	4q26	115860018	near NDST4	G	1.50E-07	0.32	0.16	0.33	2.79(0.79)	2.50E-02	4.32E-04	0.18	0.75	4.08(1.02)	2.30E-03	6.24E-05
rs2128158	8q13.2	73992654	KCNB2	A	2.28E-07	0.75	0.09	0.18	3.84(0.99)	1.23E-02	1.20E-04	0.11	0.29	4.36(1.26)	5.00E-04	5.50E-04
rs2929576	8q13.2	73986299	KCNB2	T	2.71E-07	0.78	0.09	0.17	3.84(0.99)	1.23E-02	1.18E-04	0.11	0.30	4.29(1.26)	1.50E-02	6.74E-04
rs2677485	2q24.1	156243402	near NR4A2	T	5.50E-07	0.13	0.02	0.47	11.0(2.26)	1.70E-03	1.17E-06	0.02	0.52	5.44(2.86)	7.11E-01	5.78E-02
rs6081377*	20p11.23	18724786	near DTD1	C	9.40E-07	0.98	0.37	0.28	2.40(0.62)	1.20E-02	1.11E-04	0.38	0.69	2.42(0.81)	6.42E-02	2.73E-03
rs13197942	6q25.1	150767790	IYD	T	1.34E-06	0.77	0.09	0.10	3.65(0.99)	1.77E-02	2.27E-04	0.10	0.42	4.15(1.33)	1.79E-02	1.79E-03
rs591833	1p35.2	32170477	PTP4A2	T	1.98E-06	0.22	0.14	0.58	2.69(0.85)	5.91E-02	1.62E-03	0.12	0.73	4.49(1.19)	5.49E-03	1.78E-04
rs639233	1p35.2	32145468	PTP4A2	A	2.06E-06	0.22	0.14	0.59	2.69(0.85)	5.83E-02	1.60E-03	0.12	0.54	4.49(1.20)	5.99E-03	1.89E-04
rs10769847	11p15.4	7879227	LOC283299	T	2.28E-06	0.89	0.46	0.96	2.18(0.59)	1.77E-02	2.29E-04	0.44	0.50	2.31(0.78)	7.45E-02	3.21E-03
rs682654	1p35.2	32144110	PTP4A2	T	2.36E-06	0.22	0.14	0.61	2.67(0.85)	6.33E-02	1.75E-03	0.12	0.53	4.48(1.20)	6.29E-03	1.97E-04
rs1041264*	9q34.12	133607161	RAPGEF1	T	3.07E-06	0.09	0.14	0.67	4.04(0.85)	2.10E-03	2.14E-06	0.14	0.56	1.63(1.14)	9.68E-01	1.52E-01
rs4407211	2q24.1	156214762	near NR4A2	T	3.24E-06	0.37	0.03	0.21	7.43(1.75)	6.19E-03	2.25E-05	0.03	0.30	4.84(2.30)	5.36E-01	3.59E-02
rs511395	9q21.33	87752212	NAA35	A	3.72E-06	0.05	0.03	0.01	8.55(1.74)	1.70E-03	9.21E-07	0.02	0.44	2.46(2.20)	9.99E-01	3.52E-01
rs4373300	5p13.1	38834506	Near OSMR	A	4.67E-06	0.82	0.28	0.14	2.51(0.67)	1.55E-02	1.73E-04	0.28	0.26	2.25(0.86)	1.79E-01	9.03E-03
rs4465222	1p36.32	5210118	intergenic	T	4.93E-06	0.30	0.10	0.17	4.26(1.03)	6.19E-03	2.26E-05	0.10	0.77	2.57(1.31)	6.57E-01	4.98E-02
rs11948250	5p15.32	5757234	intergenic	A	5.39E-06	0.94	0.01	0.68	10.3(2.96)	2.73E-02	5.22E-04	0.01	0.70	10.7(3.63)	7.85E-02	3.42E-03
rs13035632	2q24.1	159458544	near TANC1	C	6.11E-06	0.17	0.22	0.86	-1.98(0.72)	1.43E-01	5.58E-03	0.23	0.59	-3.6(0.94)	4.40E-03	1.40E-04
rs1752653	13q12.13	26220777	GPR12	A	6.29E-06	0.16	0.22	0.30	3.15(0.71)	4.00E-03	8.49E-06	0.20	0.08	1.49(0.95)	9.31E-01	1.20E-01
rs4905470	14q32.13	95762964	BDKRB2	A	7.41E-06	0.24	0.16	0.80	3.44(0.81)	6.19E-03	2.17E-05	0.18	0.87	1.89(1.03)	7.69E-01	6.76E-02
rs17148121	9q34.11	133493631	RAPGEF1	T	8.02E-06	0.16	0.14	0.48	3.81(0.87)	4.40E-03	1.12E-05	0.14	0.83	1.80(1.14)	9.92E-01	1.13E-01
rs10484210	4p14	36752391	Near KIAA1239	G	1.00E-05	0.37	0.21	0.18	2.16(0.71)	8.18E-02	2.53E-03	0.21	0.46	3.24(0.97)	2.22E-02	8.65E-04
rs9515034	13q32.3	108710558	MYO16	T	1.01E-05	0.22	0.05	0.86	5.74(1.33)	5.29E-03	1.76E-05	0.05	0.10	2.93(1.88)	9.30E-01	1.19E-01
rs2293585	16q24.2	88416354	Near FANCA	T	1.01E-05	0.25	0.01	0.08	11.1(2.72)	7.69E-03	4.72E-05	0.02	0.12	6.36(3.13)	5.93E-01	4.22E-02

Abbreviations: SNP, single-nucleotide polymorphism; BAND, gene band; BP, position is based on NCBI Genome Build 36.3; AL, minor allele; P-net, *p*-value for meta-analysis; Q-net, *p*-value for Cochran's Q statistic; MAF-s, Minor allele frequency of SAGE sample; HWE-s, Hardy-Weinberg equilibrium *p*-values for SAGE sample; β-sa(SE), regression coefficient and standard error for the SAGE sample; EMP2-sa, corrected empirical *p*-value for the SAGE sample generated by 100,000 permutation tests using Max (T) permutation procedure

implemented in PLINK; P -sa, p -value for the SAGE sample; MAF-c, Minor allele frequency of COGA sample; HWE-c, Hardy-Weinberg equilibrium score for COGA sample; β -c(SE), regression coefficient and standard error for the COGA sample; EMP2-co, corrected empirical p -value for the COGA sample generated by 100,000 permutation tests using Max (T) permutation procedure implemented in PLINK; P -co, p -value for the COGA sample.

* These SNPs are located at transcription factor binding sites.

Table 3

Top SNPs at 7p12.2 associated with MaxDrinks with p-value < 10⁻⁷ in the SAGE sample

SNP	BAND	BP	GENE	ALLE	P-meta	Q-meta	β-sa(SE)	P-sa	β-co(SE)	P-co
rs11575537	7p12.2	50499379	DDC	A	3.44E-05	0.0002	10.45(1.89)	3.83E-08	-2.73(2.94)	3.52E-01
rs11575549	7p12.2	50497671	DDC	A	3.49E-05	0.0002	10.45(1.89)	3.83E-08	-2.75(2.94)	3.50E-01
rs11575543	7p12.2	50498363	DDC	T	3.57E-05	0.0002	10.29(1.87)	4.15E-08	-2.62(2.89)	3.64E-01
rs11575542	7p12.2	50498481	DDC	A	4.17E-05	0.0002	10.24(1.87)	5.13E-08	-2.68(2.90)	3.55E-01
rs2190498	7p12.2	50657008	GRB10	C	5.41E-05	0.0002	10.05(1.85)	6.52E-08	-3.09(2.93)	2.93E-01
rs11575302**	7p12.2	50575188	DDC	T	2.87E-05	0.0002	10.14(1.87)	6.71E-08	-2.58(3.054)	3.99E-01
rs11575340*	7p12.2	50563965	DDC	A	4.64E-05	0.0002	10.14(1.87)	6.71E-08	-2.73(2.93)	3.52E-01
rs930707	7p12.2	50587172	DDC	A	5.27E-05	0.0004	10.14(1.87)	6.71E-08	-3.05(2.97)	3.05E-01
rs11575522	7p12.2	50502889	DDC	A	4.45E-05	0.0003	10.03(1.86)	7.79E-08	-2.64(2.94)	3.69E-01

Abbreviations: SNP, single-nucleotide polymorphism; BAND, gene band; BP, position is based on NCBI Genome Build 36.3; ALLE, minor allele; P-meta, p-value for meta-analysis; Q-meta, p-value for Cochrane's Q statistic; β-co(SE), regression coefficient and standard error for the SAGE sample; P-sa, p-value for the SAGE sample; β-co(SE), regression coefficient and standard error for the COGA sample; P-co, p-value for the COGA sample.

* The SNP is located at a transcription factor binding site;

** The SNP is located at an exonic splicing silencer or an exonic splicing enhancer.

Table 4SNPs associated with MaxDrinks in the OZALC sample (P -value < 0.05)

SNP	BAND	BP	GENE	ALLE	MAF	HWE	N	P-PBAT	FDR	P-meta	P-sa	P-co
rs1402071	2q24.1	156467537	near NR4A2	T	0.28	0.36	459	0.0019	0.38	5.19E-01	4.84E-01	8.85E-01
rs10497151	2q24.1	156405342	near NR4A2	C	0.08	0.14	234	0.0033	0.38	9.62E-01	9.07E-01	9.35E-01
rs10497172	2q24.1	156678543	near NR4A2	G	0.05	0.99	154	0.0051	0.39	3.06E-01	4.69E-01	4.55E-01
rs1113060	2q24.1	156656293	near NR4A2	T	0.20	0.09	407	0.0127	0.39	4.04E-01	9.50E-01	1.86E-01
rs1092915	2q24.1	156758045	near NR4A2	A	0.11	0.73	282	0.0150	0.39	8.53E-01	9.37E-01	8.38E-01
rs1519805	2q24.1	156689940	near NR4A2	G	0.19	0.61	382	0.0190	0.39	5.62E-01	9.71E-01	3.56E-01
rs4664804	2q24.1	156816639	near NR4A2	C	0.11	0.83	281	0.0195	0.39	9.64E-01	9.59E-01	8.84E-01
rs2677468	2q24.1	156249416	near NR4A2	T	0.44	0.99	499	0.0201	0.39	3.72E-01	5.64E-01	4.73E-01
rs1914648	2q24.1	156273051	near NR4A2	G	0.34	0.75	488	0.0230	0.40	3.10E-01	7.44E-01	2.07E-01
rs12471739	2q24.1	156720471	near NR4A2	A	0.36	0.41	469	0.0264	0.40	5.63E-01	4.98E-01	9.49E-01
rs1283760	2q24.1	156261694	near NR4A2	A	0.34	0.65	490	0.0364	0.49	2.55E-01	7.12E-01	1.58E-01
rs1004791	2q24.1	156336395	near NR4A2	T	0.33	0.98	487	0.0390	0.49	6.92E-01	5.61E-01	9.17E-01
rs2620418	4q26	116143994	NDST4	C	0.31	0.30	502	0.0103	0.39	2.29E-01	7.02E-01	1.43E-02
rs11938588	4q26	116032462	NDST4	A	0.11	0.82	300	0.0203	0.39	7.70E-01	8.68E-01	7.90E-01
rs6868097	5q23.1	118273092	DTWD2	C	0.33	0.89	474	0.0209	0.39	7.01E-01	5.87E-01	9.27E-01
rs2929576	8q13.2	73986299	KCNB2	T	0.10	0.74	261	0.0142	0.39	2.71E-07	1.18E-04	6.74E-04
rs2958414	8q13.3	73849754	KCNB2	G	0.08	0.26	242	0.0250	0.40	7.83E-01	3.72E-01	4.55E-01
rs1822059	8q13.3	73828294	KCNB2	A	0.08	0.23	242	0.0318	0.46	6.93E-01	3.50E-01	5.52E-01
rs4541976	8q13.2	73958102	KCNB2	C	0.09	0.97	263	0.0417	0.50	8.12E-01	9.88E-01	6.98E-01
rs7829474	8q13.2	73957878	KCNB2	A	0.09	0.97	264	0.0477	0.54	8.31E-01	9.79E-01	6.86E-01

Abbreviations: SNP, single-nucleotide polymorphism; BAND, gene band; BP, position is based on NCBI Genome Build 36.3; ALLE, minor allele; MAF, Minor allele frequency for OZALC sample; HWE, Hardy-Weinberg equilibrium score for OZALC sample; P-PBAT, p -value of the replication study for OZALC sample; P-meta, p -value for meta-analysis; FDR, the false discovery rate for the p -value; P-sa, p -value for the SAGE sample; P-co, p -value for the COGA sample.

Table 5*Cis*-regulatory effects of risk SNPs on transcript expression

SNP	Gene	Tissue	Beta	P
rs11575522	DDC	Brain	-12.3	0.048
rs11575542 (Arg462Gln)	DDC	Brain	-13.1	0.016
rs11575522	DDC	PBMC	-13.4	0.038
rs11575542 (Arg462Gln)	DDC	PBMC	-9.6	0.050
rs6868097	DTWD2	PBMC	6.4	0.050
rs2958414	KCNB2	Brain	35.4	0.018
rs11938588	NDST4	PBMC	0.9	0.029
rs12471739	NR4A2	PBMC	-179.5	0.048
rs1041264*	RAPGEF1	Brain	2.8	0.029
rs17148121	RAPGEF1	Brain	3.1	0.018

*
This SNP is located at a transcription factor binding site.