Genetic variants in the CPNE5 gene are associated with alcohol dependence and obesity in Caucasian populations

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A B S T R A C T

Alcohol addiction may increase the risk of obesity due to shared genetic components. The Copine V (CPNE5) gene is involved in Ca^{2+} binding and may play an important role in the development of the central nervous system. This study tested the genetic associations of 77 single-nucleotide polymorphisms (SNPs) within the CPNE5 gene with alcohol dependence (AD) and obesity using a Caucasian sample — The Study of Addiction — Genetics and Environment (SAGE) sample (1066 AD cases and 1278 non-AD controls; 422 obese cases and 1395 non-obese controls). The Marshfield sample (1442 obese cases and 2122 non-obese controls) was used for replication of obesity. Multiple logistic regression analysis was performed using the PLINK software. In the SAGE sample, we identified 10 SNPs associated with AD and 17 SNPs associated with obesity (p < 0.05). Interestingly, 6 SNPs (rs9986517, rs9470387, rs3213534, rs10456444, rs3752480, and rs9470386) were associated with both AD (OR = 0.77, 0.77, 0.83, 0.84, 0.79 and 1.14, respectively; p = 9.72 × 10^{-3}, 1.1 × 10^{-4}, 4.09 × 10^{-3}, 5.26 × 10^{-3}, 1.59 × 10^{-2}, and 3.81 × 10^{-2}, respectively) and obesity (OR = 0.77, 0.77, 0.78, 0.77, 0.68 and 1.18, respectively; p = 2.74 × 10^{-3}, 2.69 × 10^{-3}, 2.45 × 10^{-3}, 1.01 × 10^{-2}, 5.18 × 10^{-3} and 3.85 × 10^{-2}, respectively). In the Marshfield sample, rs3752480 was associated with obesity (p = 0.0379). In addition, four SNPs (rs9886517, rs10456444, rs7763347 and rs4714010) showed associations with obesity in the meta-analysis using both samples (p = 0.00493, 0.0274, 0.00346, and 0.0141, respectively). These findings provide the first evidence of common genetic variants in the CPNE5 gene influencing both the AD and obesity; and will serve as a resource for replication in other populations.

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1. Introduction

Alcohol consumption is the third leading risk factor globally for disease burden, and harmful use of alcohol leads to 2.5 million deaths worldwide every year (WHO, 2010). In the United States (US), 71% of the general US aged 18 or older reported that they drank in the past year. One quarter (24.6%) reported engaging in binge drinking, and 7.1% reported heavy drinking in the past month (SAMHSA, 2012). Based on the 2009–2010 National Health and Nutrition Examination Survey (NHANES) data, the age-adjusted

obesity prevalence among US adults 20 years and older was 35.7% (Flegal et al., 2012), with the absolute numbers of obese individuals globally projected to surpass 1.12 billion by 2030 (Kelly et al., 2008). Previous epidemiological data suggest that moderate alcohol intake may protect against obesity, particularly in women, whereas higher consumption including binge-drinking may increase the risk of obesity (Wilson, 2010; Yeomans, 2010; Wakabayashi, 2014). Results are inconsistent, however. For example, one study suggested that heavy drinking was not related to obesity (Adachi et al., 2000), with another study reporting frequent drinking was associated with reduced odds of obesity (Rohrer et al., 2005). Using the 1988–1994 NHANES data in the non-smoking US adult population, the odds of overweight and obesity were significantly higher among binge drinkers and those consuming four or more drinks/day. However, those who reported
drinking one or two drinks per day, or less than five drinks per week, had decreased odds of obesity (Ariif and Rohrer, 2005). One recent study using the NHANES registry of 1999–2002 suggested potential gender differences in the link between alcohol consumption and obesity. Binge drinking was associated with significantly higher odds of obesity, for both males and females, however, moderate drinking (3 drinks/day in females and 4 drinks/day in males) was associated with increased odds of obesity in females, but decreased odds of obesity in males (Chakraborty, 2014).

Alcohol dependence (AD) is a psychiatric diagnosis evidenced by physical or psychological dependence on alcohol. According to the Diagnostic and Statistical Manual of Mental Disorders (DSM) IV criteria for AD, at least three out of seven of the following criteria must be manifest during a 12-month period: tolerance, withdrawal symptoms, use in larger amounts or for longer periods than intended, persistent desire, loss of control, reduction or cessation of social, occupational and recreational pursuits, continued use despite knowledge of alcohol-related harm. (http://www.alcoholcostcalculator.org/business/about/dsm.html). Among American adults, approximately 12% have had an AD problem in their lifetime, with 4% classified as having an AD problem in the previous 12-months (Hasin et al., 2007). Family, twin, and adoption studies of the CPNE5 gene and environment factors, as well as their interactions, all contribute to the development of AD, with a heritability of more than 0.5 (Heath et al., 1997; Schuckit, 2000; Goldman et al., 2005; Bierut et al., 2010). Recently, several genome-wide association studies (GWAS) have been completed and a number of candidate genes have been found to be associated with the risk of AD and alcohol consumption (e.g., Bierut et al., 2010; Edenberg et al., 2010; Schumann et al., 2011; Wang et al., 2011; Zuo et al., 2012; Gelernter et al., 2014).

Binge eating disorder, and overeating as an addictive disorder, are also psychiatric disorders in DSM-V and are often comorbid with obesity (James et al., 2004; Volkow and O’Brien, 2007; American Psychiatric Association, 2013). Alcohol addiction may also be comorbid with obesity, and those with AD may be at increased risk of obesity due to shared genetic components (Wang et al., 2013).

The Copine V (CPNE5) (also known as CPNS5, COPN5) gene is located at 6p21.2 (Creuzet et al., 1998; Tripidis et al., 1998). Copines are a family of calcium-dependent lipid-binding proteins comprised of 2 N-terminal C2 domains (C2Ds) and a C-terminal A domain. The C2Ds contain aspartate residues important for calcium and phospholipid binding (Rasmeyer et al., 2008). The CPNE5 is one of several genes that encode a calcium-dependent protein containing two N-terminal type II C2 domains and an integrin A domain-like sequence in the C-terminus. A recent study showed that CPNE5 is expressed in both neural progenitor cells and the differentiated neurons during the neural development, suggesting that CPNE5 might play an important role in the development of the central nervous system (Ding et al., 2008). Although alcohol’s effects on the central nervous system, including neuro-cognitive deficits, neuronal injury and neurodegeneration, are well documented, the biological and genetic mechanisms remain elusive (Mukherjee, 2013). Hence, CPNE5 is a suitable candidate gene for study in AD. In the present study, we hypothesized that CPNE5 plays a role in AD, with some genetic variants within the CPNE5 gene potentially associated with both AD and obesity. This study explored the associations of 77 single-nucleotide polymorphisms (SNPs) within the CPNE5 gene with both AD and obesity. This study compared the associations of 77 single-nucleotide polymorphisms (SNPs) within the CPNE5 gene with both AD and obesity. The study of the association of the CPNE5 gene with both AD and obesity in The Study of Addiction—Genetics and Environment (SAGE) sample (1066 AD cases and 1278 non-AD controls, 422 obese cases and 1395 non-obese controls). The Marshfield sample (1442 obese cases and 2122 non-obese controls) was used for replication of obesity.

2. Materials and methods

2.1. Samples

2.1.1. The SAGE sample

SAGE is a comprehensive genome-wide association study (GWAS) of approximately 4000 unrelated subjects of European and African-American descent. It was funded as part of the Gene Environment Association Studies (GENEVA) initiative supported by the National Human Genome Research Institute (dbGaP study accession phs000092.v1.p1). Cases used for this report were 1066 Caucasian subjects with the primary phenotype of a lifetime history of AD using DSM-IV criteria (Bierut et al., 2010). Controls consisted of 1278 Caucasian subjects who had used alcohol, but never diagnosed as having AD or drug dependence (DD) (due to the likely genetic overlap between AD and DD). A subset of 1817 individuals had height (in inches) and weight (in pounds), with body mass index (BMI) calculated by dividing weight in pounds by height in inches squared and multiplying by a conversion factor of 703. Obesity was determined as a body mass index (BMI) > 30 (WHO, 1998). The SAGE sample contains about 1 million Illumina SNPs. Samples were genotyped at the Johns Hopkins Center for Inherited Disease Research (CIDR). Genotyping was performed using Illumina HumanMv1_1C BeadChips and the Illumina Infinium II assay protocol (Gunderson et al., 2006). Allele cluster definitions for each SNP were determined using Illumina BeadStudio Genotyping Module version 3.1.14 and the combined intensity data from the samples. A SNP call rate of 98% was required. Within the CPNE5 gene, 77 SNPs in the SAGE sample were available.

2.1.2. The Marshfield sample

The Marshfield sample produced publicly available data from “A Genome-Wide Association Study on Cataract and HDL in the Personalized Medicine Research Project Cohort” - Study Accession: phs000170.v1.p1 (dbGaP). The primary goals of this project are to develop and validate electronic phenotyping algorithms, to accurately identify cases and controls while maintaining a positive predictive value (PPV) of >95%, and to conduct a genome-wide association study that advances the understanding of two specific yet interrelated disease states, while simultaneously engaging the community in these research efforts. The details about these subjects were described elsewhere (McCarty et al., 2005, 2008). While AD status was not available in the Marshfield sample, for the 3564 Caucasian individuals available, all of had height (in centimeters) and weight (in kilograms), allowing for calculation of BMI and determination of obesity status. Genotyping data using the ILLUMINA Human660W-Quad_v1.1_A was available for the entire sample, with samples genotyped at the Johns Hopkins Center for Inherited Disease Research (CIDR). Within the CPNE5 gene, 59 SNPs were available.

2.2. Statistical analyses

HAPLOVIEW software was used for quality control (Barrett et al., 2005). First, Hardy–Weinberg equilibrium (HWE) was tested for all of the SNPs in controls. Then, minor allele frequency (MAF) was determined for each SNP. Third, the linkage disequilibrium (LD) structure based on D’ values was constructed. For the SAGE sample, logistic regression analysis of AD and obesity separately, adjusted for age and sex, was performed. For the Marshfield sample, logistic regression analysis of obesity adjusted for age and sex, was conducted. The asymptotic p-values for the logistic regression models were calculated while the odds ratio (OR) and its standard error were estimated using PLINK v1.07 ( Purcell et al., 2007). Since the two samples shared the same genotyping platform, results for
obesity were directly meta-analyzed by combining the separate results of obesity from two samples (OR and standard error of OR) into overall effects. For this meta-analysis of the two datasets, the basic meta-analysis function in PLINK was applied. Fixed-effect meta-analysis p-value and fixed-effect ORs were estimated. The between-study heterogeneity was tested by the \( \chi^2 \)-based Cochrane's Q statistic (Higgins and Thompson, 2002). Haplotype analyses of AD and obesity were separately performed in the SAGE sample using the PLINK software.

### 3. Results

#### 3.1. Genotype quality control and descriptive statistics

All 77 SNPs in the SAGE sample and 59 SNPs in the Marshfield sample were in HWE in the controls (\( p > 0.01 \)). Participant characteristics for two samples are presented in Table 1. There were more obese females than males in both cases and controls in both samples, as well as within AD controls in the SAGE sample. However, there were more males than females among AD cases in the SAGE sample. The mean ages for the Marshfield sample were substantially higher than those in the SAGE sample.

#### 3.2. Association with alcohol dependence in the SAGE sample

Single marker analysis showed that 10 SNPs within the CPNE5 gene in the SAGE sample were associated with AD (\( p < 0.05 \)) (Table 2). The top three SNPs showing significant associations with AD were rs9986517, rs9470387, and rs929051 (OR = 0.77, 95% CI = 0.68–0.88 with \( p = 9.72 \times 10^{-5} \); OR = 0.77, 95%CI = 0.68–0.88 with \( p = 1.1 \times 10^{-4} \); and OR = 1.24, 95%CI = 1.10–1.4 with \( p = 5.18 \times 10^{-4} \), respectively). Results remained significant after Bonferroni correction (\( z = 0.05/77 = 6.49 \times 10^{-5} \)). Haplotype analyses showed the T-C haplotype from rs9986517 and rs1064827 (\( D' = 1.0 \) between two SNPs in Fig. 1) was significantly associated with AD (\( p = 9.79 \times 10^{-5} \)) (Table 3).

#### 3.3. Association with obesity in the SAGE sample

A total of 17 SNPs associated with obesity were identified in the SAGE sample (\( p < 0.05 \)) (Table 2). The most significant SNP for obesity was rs10456444 followed by rs3213534 and rs9470387. Haplotype analysis showed the C-C haplotype from rs1064827 and rs10456444 (\( D' = 1.0 \) between two SNPs in Fig. 1) was significantly associated with obesity (Table 4).

#### 3.4. Replication study of obesity and meta-analysis

In the Marshfield sample, single marker analysis showed that rs3752480 was associated with obesity (\( p = 0.0379 \)) (data not shown). We then focused on the 21 SNPs associated with AD or obesity in the SAGE sample (Table 2). Among the 21 SNPs in Table 2, 16 SNPs overlapped in both samples. Four SNPs (rs929051, rs7763347, rs763046, and rs4714010) were associated with obesity in the Marshfield sample with borderline significance (0.05 \( \leq p \leq 0.10 \)). In addition, four SNPs (rs9986517, rs10456444, rs7763347 and rs4714010) were associated with obesity in the meta-analysis of the SAGE and Marshfield samples (Table 2).

### 3.5. Shared SNPs among alcohol dependence and obesity

Table 2 shows that 6 SNPs in the SAGE sample (rs9986517, rs9470387, rs3213534, rs10456444, rs3752482, and rs9470386) were associated with both AD and obesity. In addition, 2 SNPs (rs9986517 and rs10456444) associated with AD in the SAGE sample also revealed associations with obesity in the meta-analysis of the two samples (OR \( = 0.88 \) and 0.91, respectively; \( p = 4.93 \times 10^{-3} \) and 2.74 \( \times 10^{-2} \), respectively). The allele effects of these 6 SNPs on AD and obesity all have the same directions.

### 4. Discussion

To our knowledge, no studies investigating associations of CPNE5 polymorphisms with comorbid AD and obesity have been published. In this study, we identified 10 SNPs associated with AD and 17 SNPs associated with obesity in the SAGE sample. Interestingly, 6 SNPs (rs9986517, rs9470387, rs3213534, rs10456444, rs3752482, rs9470386) were associated with both AD and obesity. In addition, 2 SNPs (rs9986517 and rs10456444) also showed associations with obesity in the meta-analysis of two samples. Haplotype analyses further supported the single-marker analysis results of AD and obesity in the SAGE sample. In addition, some of the obesity results in the SAGE sample were replicated in the Marshfield sample and meta-analysis.

The CPNE5 gene is one of the genes in a family of calcium-dependent lipid-binding proteins comprised of 2 N-terminal C2 domains (C2Ds) and a C-terminal A domain. It has been reported that the C2 domains of CPNE5 are capable of Ca\(^{2+}\) binding (Tomsig et al., 2003; Cho and Stahelin, 2006; Hurley, 2006), which plays a key role in the development of the nervous system. For example, Ca\(^{2+}\) is a second messenger in neuronal signal transduction and is implicated in many processes, especially apoptosis (Nutt et al., 2002). Studies have demonstrated that CPNE5 transcripts are abundant during the development of embryonic mouse brain, and may play an important role during neurogenesis (Ding et al., 2008). A recent study showed that CPNE5 is implicated in synaptic function, behavioral plasticity, or resource constraint and is moderately expressed in the adult mouse striatum (D’Amours et al., 2011). Furthermore, previous work reveals a possible association of alcohol tolerance with increased synaptic Ca\(^{2+}\) sensitivity (Lynch and Littleton, 1983), with alcohol potentially leading to calcium ion (Ca\(^{2+}\)) overload (Altura and Altura, 1994). Further studies reveal

### Table 1

Descriptive characteristics of cases and controls.

<table>
<thead>
<tr>
<th></th>
<th>SAGE sample</th>
<th>Marshfield sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AD (^a)</td>
<td>Control</td>
</tr>
<tr>
<td>Number</td>
<td>1066</td>
<td>1278</td>
</tr>
<tr>
<td>Sex, N (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>646 (61%)</td>
<td>376 (29%)</td>
</tr>
<tr>
<td>Females</td>
<td>420 (39%)</td>
<td>902 (71%)</td>
</tr>
<tr>
<td>Age, years</td>
<td>Mean ± SD</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>38.1 ± 9.9</td>
<td>38.6 ± 9.4</td>
</tr>
<tr>
<td>Range</td>
<td>18–77</td>
<td>18–65</td>
</tr>
</tbody>
</table>

\(^a\) AD: Alcohol dependence.
that AD can develop at the cellular level, with changes in calcium homeostasis (Nagy, 2000), while alcohol-induced apoptosis may contribute to alcohol-induced brain-vascular damage and stroke (Li et al., 2004). Recent research shows that Ca\textsuperscript{2+} may play a role in pancreatic cell death and acute pancreatitis induced by alcohol metabolites (Criddle et al., 2006; Gerasimenko et al., 2009).

In the present study, 10 SNPs were found to be associated with AD, with 2 of these also associated with both AD and obesity in the SAGE sample, with 2 of these also associated with obesity in the Marsh field sample. Our results and our present results suggested that Ca\textsuperscript{2+} may be one of the mechanisms linking AD and obesity.

Previous animal models have shown that Ca\textsuperscript{2+} may be related to the development of obesity (Chan, 1995; Kim et al., 1996). Other studies suggest that regulation of intracellular Ca\textsuperscript{2+} ([Ca\textsuperscript{2+}]\textsubscript{i}) plays a key role in obesity, insulin resistance and hypertension (Zemel, 1998; Zemel and Miller, 2004). Furthermore, it has been recently reviewed that Ca\textsuperscript{2+} may induce apoptosis of cancer cells and adipocytes, resulting in decreasing tumor size and long-term reduction in adipose tissue mass (Sergeev, 2013, 2014). In this study, we performed the first genetic association study and meta-analysis of obesity for CPNE5 gene, and identified 17 SNPs associated with obesity in the SAGE sample and 4 SNPs associated with obesity in the meta-analysis of the SAGE and Marshfield samples. Our results indicate that the CPNE5 gene might also play a potential role in obesity.

Previous epidemiological studies reveal heavy drinking or binge-drinking may increase the risk of obesity (Arif and Rohrer, 2005; Wilson, 2010; Yeomans, 2010; Chakraborty, 2014; Wakabayashi, 2014). Furthermore, some previous studies also support a link between substance use, abuse or dependence, with obesity, but results are inconsistent (Barry et al., 2009; Grucca et al., 2010; Pickering et al., 2011). For example, a relationship between being overweight and nicotine and alcohol dependence or abuse was found among men, but not among women (John et al., 2005). In addition, obesity was associated with psychiatric disorders and suicidal behavior in a Canadian sample (Mather et al., 2009). Another study suggested that depression, obesity and alcohol use disorders were interrelated conditions for women, but a greater understanding of reasons underlying the co-occurrence of these conditions would benefit prevention and intervention efforts (McCarty et al., 2009). In the present study, 6 SNPs were associated with both AD and obesity in the SAGE sample, with 2 of these also associated with obesity in the meta-analysis. The effects of these 6 SNPs had the same directions: the minor alleles of 5 SNPs showed protective effect on both AD and obesity while the other one showed protective effect on both AD and obesity. These results provide evidence that the CPNE5 gene has a pleiotropic effect on AD and obesity. Our results also support previous studies that obesity or uncontrolled eating disorder may share some pathways with excessive ethanol consumption or AD and addiction (Thiele et al., 2003; Tomasi and Volkow, 2013; Lichenstein et al., 2014). Previous findings and our present results suggested that Ca\textsuperscript{2+} may be one of the mechanisms linking AD and obesity.

The current study has a number of strengths. First, to our knowledge, this is the first candidate gene study which investigated the associations between CPNE5 polymorphisms and comorbidities of AD and obesity. Second, our sample sizes were relatively large and the two samples were ethnically homogeneous. Third, we examined 77 SNPs within the CPNE5 gene in the SAGE sample, and used 59 SNPs in the Marshfield sample to replicate our findings. Fourth, we implemented a meta-analysis to increase the study power and precision by combining two study samples. Finally, we were able to detect pleiotropic effects of CPNE5 gene on two complex diseases – AD and obesity.
The current study is not without limitations, however. First, only one sample had information on AD. Second, this study focused on obesity classified simply as BMI \( \geq 30 \). Additional research should compare subcategories of obesity such as Class I obesity (BMI = 30–34.9), Class II obesity (BMI = 35–39.9), and Class III obesity (BMI \( \geq 40 \)), as differences may exist in observed findings. Third, our current findings might be subject to type I error due to the number of comparisons made. Fourth, unequal distribution of gender across AD and obesity groups may have impacted findings. Finally, the mean ages for the Marshfield were higher than those in the SAGE samples. Replication of current findings in additional samples is clearly needed.

5. Conclusion

These findings provide the first evidence of genetic variants in the CPNE5 gene influencing both AD and obesity. Results of the current study could serve as a resource for replication in other populations. Future functional studies within CPNE5 may help to better characterize the genetic architecture of these two complex diseases. The findings also provide evidence for the utility of potential joint intervention and prevention efforts among patients with AD and obesity.

Conflicts of interest

All authors have reported no financial interests or potential conflicts of interest.
Contributors

Kesheng Wang and Yue Pan managed the literature searches and analyses, edited the references, and wrote the draft of the manuscript. Lingjun Zuo and Xingguang Luo offered critical guidance in the design of this study and provided a substantive review of the manuscript. Changchun Xie offered critical guidance on the statistical analysis and contributed for statistical expertise and improvement of the manuscript. Kesheng Wang designed the study and improved the manuscript. All authors read and approved the manuscript.

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This study was approved by the Internal Review Board (IRB), East Tennessee State University.

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