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ORIGINAL RESEARCH

Associations of *BRAP* polymorphisms with the risk of alcohol dependence and scores on the Alcohol Use Disorders Identification Test

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Background: Alcohol dependence (AD) is a common disorder that is influenced by genetic as well as environmental factors. A previous genome-wide association study (GWAS) of the Korean population performed by our research group identified a number of genes, including *BRCA1-associated protein* (*BRAP*) and *protein arginine methyltransferase 8* (*PRMT8*), as novel genetic markers of AD.

Methods: The present investigation was a fine-mapping follow-up study of 459 AD and 455 non-AD subjects of Korean descent to determine the associations between *BRAP* and *PRMT8* polymorphisms and AD. The Alcohol Use Disorders Identification Test (AUDIT) was administered to screen for the degree of AD risk in the subjects and 58 genetic variants, 5 for *BRAP* and 53 for *PRMT8*, were genotyped for subsequent association analyses.

Results: In the present case–control analysis, *BRAP rs3782886* showed the most significant association signal with a risk of AD (P=1.29×10^{-16}, P_{corr} =7.74×10^{-16}, OR =0.19). There were also significant differences in the overall and subcategory scores for the *BRAP* genetic variants, including *rs3782886* (P=9.94×10^{-31}, P_{corr} =5.96×10^{-30} at *rs3782886* for the overall AUDIT score). However, the genetic effects of *PRMT8* polymorphisms observed in our previous GWAS were not replicated in the present study (minimum P=0.0005, P_{corr} >0.05, OR =0.30 at *rs4766139* in the recessive model). Furthermore, the single-nucleotide polymorphisms of *PRMT8* were not associated with the overall and subcategory AUDIT scores.

Conclusion: The present findings suggest that the genetic variants of *BRAP* may contribute to a predisposition for an alcohol use disorder.

Keywords: alcohol dependence, AUDIT, genome-wide association study, single-nucleotide polymorphism, BRAP

Introduction

Alcohol dependence (AD) is a severe psychiatric disorder with a multifactorial etiology that includes complex gene-to-gene and gene-to-environment interactions.[1–3] Adoption and twin studies conducted to clarify the effects of genes in this etiology have revealed that genetic factors comprise 50%–60% of the heritability of AD susceptibility.[4,5] Additionally, adoptees are more similar to their biologic parents than their adoptive parents in terms of AD susceptibility[6,7] and the higher concordance for AD susceptibility between twins is derived from shared genetic components.[8] In fact, several candidate studies assessing the risk loci for AD were designed to target gene variants related to alcohol metabolism or neurobiology.[9–13]

Recently, a number of genome-wide association studies (GWASs) have investigated genetic markers of AD, including the genomic region of chromosome

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4q22-q32, which includes alcohol dehydrogenase (*ADH*) cluster genes.[14–17] Furthermore, a recent GWAS of a Korean AD cohort revealed that three chromosomal regions are associated with AD, including the *ADH* gene cluster and *ALDH2*, which participate in alcohol metabolism (minimum $P=6.46\times10^{-8}$, OR =2.73 at *ADH7 rs10516441* of the *ADH* gene cluster and $P=8.42\times10^{-8}$, OR =0.22 at *ALDH2 rs671*). The genetic effects of the *ADH* gene cluster were also replicated in a Korean population (minimum $P=2.63\times10^{-21}$ at *ADH1B rs1229984*). In addition to genes related to alcohol metabolism, genes known to participate in neurodevelopment, such as *BRCA1-associated protein* (*BRAP*) and *protein arginine methyltransferase 8* (*PRMT8*), have multiple association signals with the risk of AD ($P=4.65\times10^{-6}$, OR =0.31 at *BRAP rs3782886* and $P=1.77\times10^{-5}$, OR =1.96 at *PRMT8 rs876594*).[18] Based on the polygenic hypothesis of AD pathophysiology, it is possible that multiple genetic loci associated with neurobiologic pathways could be associated with the risk of AD. Thus, the present investigation conducted follow-up replication studies of our previous GWAS of a Korean cohort with AD to identify associations between the risk of AD and novel candidate genes other than those related to alcohol metabolism.

Methods

Subject recruitment and the Alcohol Use Disorders Identification Test (AUDIT)

The present study recruited 914 individuals of Korean descent from Hangang Sacred Heart, Keyo, Dasarang, KARF, and Humanity and Youth Rehabilitation Hospitals. Of these individuals, 459 were alcoholic subjects and 455 were nonalcoholic subjects; the nonalcoholic subjects were recruited from the industrial medical center of Hangang Sacred Heart Hospital. All subjects enrolled in this study underwent inpatient therapy for >30 days due to their drinking problems and the patients who comprised a subgroup in our previous study did not have major medical or comorbid psychiatric illnesses other than an alcohol-related disorder.[11,19] AD was diagnosed clinically with a semi-structured interview based on the guidelines of the *Diagnostic and Statistical Manual of Mental Disorders* IV[20] by skilled psychiatrists as well as on information provided by their caregivers; diagnostic validity was high because all subjects were hospitalized in alcohol-related hospitals. Most of the healthy controls were nondrinkers, although some were occasional light drinkers as revealed by a drinking habit questionnaire. Subjects who had first-degree relatives with major psychiatric disorders, including schizophrenia, mood disorders, and/or substance abuse disorders other than nicotine dependence, were excluded from the present analyses. The study protocol was approved by the institutional review board of each hospital. All participants provided written informed consent, and that this study was conducted in accordance with the Declaration of Helsinki.

The AUDIT was administered to screen for the degree of AD risk in the subjects.[21] The AUDIT consists of ten items and is often used in Asian populations, including the Korean population. This tool includes three domain structures: items 1–3 measure alcohol consumption, items 4–6 assess AD, and items 7–10 evaluate alcohol-related harm.[22,23] All items are equally weighted, the scores range from 0 to 4, and the total AUDIT score is determined by summing all subcategory scores; a higher AUDIT score is indicative of a higher risk in each category.

Genotyping of the *BRAP* and *PRMT8* polymorphisms

To assess genomic DNA precisely, a DNA quantification analysis was performed using Quanti-iT PicoGreen fluorescence dye (Molecular Probes, Eugene, OR, USA). The quantification reactions were performed according to the manufacturer's instructions (Manual No: MP0758) and the concentration of each type of genomic DNA was measured with a Fluorescence Reader (VICTOR2 fluorometer; Perkin Elmer, CA, USA). Candidate single-nucleotide polymorphisms (SNPs) of *BRAP* and *PRMT8* were selected from among Japanese and Han Chinese genotype data using the 1,000 Genomes database (http://browser.1000genomes.org/index.html) based on the following conditions: 1) minor allele frequency (MAF) >5%; 2) linkage disequilibrium (LD) status based on an LD coefficient (r^2) >0.98; 3) positions within the gene; and 4) amino acid changes. A total of 58 SNPs (5 from *BRAP* and 53 from *PRMT8*) were genotyped in the 459 alcoholic subjects and 455 nonalcoholic subjects using the Illumina Golden Gate genotyping system at a multiplex level.[24] The genotyping quality score for retaining data was set to 0.25 and SNPs that did not satisfy the following criteria were excluded: 1) a minimum call rate of 95% and 2) no duplicate errors.

Statistical analysis

The LD was obtained using Haploview v4.2 software (http://www.broadinstitute.org/mpg/haploview) based on assessments of Lewontin's D′ ($|D'|$) and the r^2 between all pairs of biallelic loci.[25] Haplotypes were determined using PHASE v2.0 software[26] and comparisons of the genotype distributions between alcoholic and nonalcoholic subjects were carried out with a logistic regression model adjusted for age (continuous value) and sex (male =0, female =1) using SAS,

version 9.4 (SAS Institute Inc., Cary, NC, USA). Associations between SNPs and AUDIT scores were also calculated using a linear regression model adjusted for age and sex. Statistical power of single associations was calculated using the Power for Genetic Association Analyses software,[27] with false positive rate of 5%, disease prevalence of 4%,[28] given MAFs and sample sizes, and assuming a relative risk of 1.5. Corrected *P*-values for multiple testing were calculated using the Bonferroni correction method.

Results

The present study included a total of 914 subjects who were categorized as either AD (n=459, mean age =47.37 years, range =21–80 years, 410 males and 49 females) or non-AD (n=455, mean age =44.21 years, range =20–79 years, 351 males and 104 females; Table 1); there were no significant differences between the AD and non-AD subjects in terms of age or sex. The degree of AD risk was estimated using AUDIT scores.

Genotyping and haplotype analyses of *BRAP* and *PRMT8* SNPs

A total of 58 SNPs (5 from *BRAP* and 53 from *PRMT8*) were genotyped in all subjects. The position, LD, and haplotype information of the investigated SNPs are shown in Figure S1. The *BRAP* and *PRMT8* polymorphisms investigated in the present study were parsed into 1 LD block and 11 LD blocks, respectively. Not all haplotypes were selected for subsequent analyses because some haplotypes were tagged by SNPs on each gene.

Association analyses of *BRAP* and *PRMT8* SNPs with AD

Logistic regression analyses were conducted to investigate the associations between *BRAP* and *PRMT8* genetic variants and the risk of AD. In the case–control analysis, three genetic variants of *BRAP* (*rs847895*, *rs3782886*, and *rs3803171*) were associated with the risk of AD (minimum $P=1.29\times10^{-16}$, OR =0.19 at *rs3782886*) under the codominant model (Table 2). Of the *PRMT8* SNPs, one genetic variant (*rs12581829*) was marginally associated with the risk of AD under the codominant model ($P=0.02$, OR =0.72) and two SNPs (*rs4766138* and *rs4766139*) showed nominal associations with the risk of AD under the recessive model ($P=0.002$, OR =0.33 and $P=0.0005$, OR =0.30, respectively; Table S1). However, the statistical significance of the *PRMT8* SNPs disappeared after corrections for multiple analyses were performed.

Associations of *BRAP* and *PRMT8* SNPs with the degree of AD risk

To screen for the degree of risk of AD, additional association analyses between the AUDIT score and genetic variants of *BRAP* or *PRMT8* that showed associations with the risk of AD were conducted. For *BRAP*, three SNPs (*rs3803171*, *rs3782886*, and *rs847895*) and one haplotype (*ht1*) were significantly associated with the overall AUDIT score (minimum $P=9.94\times10^{-31}$ and minimum $P_{corr}=5.96\times10^{-30}$ at *rs3782886*) and three SNPs had significant association signals with alcohol use disorders (minimum $P=3.30\times10^{-46}$ and $P_{corr}=1.98\times10^{-45}$ at *rs3782886* for alcohol consumption, minimum $P=1.95\times10^{-17}$ and $P_{corr}=1.17\times10^{-16}$ at *rs3782886* for AD, and minimum $P=3.89\times10^{-22}$ and $P_{corr}=2.34\times10^{-21}$ at *rs3782886* for alcohol-related harm) based on the AUDIT scoring (Table 3). The strengths of the associations between the *BRAP* SNPs and alcohol use disorders were greater in non-AD subjects. Additionally, *BRAP rs3782886* was strongly associated with the overall AUDIT score ($P=1.40\times10^{-24}$ and $P_{corr}=8.39\times10^{-24}$) and the subcategories of the AUDIT ($P=6.46\times10^{-32}$ and $P_{corr}=3.87\times10^{-31}$ for alcohol consumption, $P=2.59\times10^{-7}$ and $P_{corr}=1.56\times10^{-6}$ for AD, and $P=3.88\times10^{-10}$, and $P_{corr}=2.32\times10^{-9}$ for alcohol-related harm; Table 4).

The association analysis of the *PRMT8* SNPs revealed that individuals with three SNPs (*rs4766138*, *rs4766139*,

Table 1 Clinical profiles of study subjects

Description	AD	Non-AD	*P*-value
N	459	455	
Age (year; mean-range)	47.37 (21–80)	44.21 (20–79)	<0.001[a]
Gender (M/F)	410/49	351/104	<0.001[b]
AUDIT			
Overall	27.90±7.39 (0–40)	8.58±6.36 (0–29)	<0.001[a]
Alcohol consumption	10.23±2.10 (0–12)	5.73±3.46 (0–12)	<0.001[a]
Alcohol dependence	7.37±3.26 (0–12)	0.99±1.50 (0–8)	<0.001[a]
Alcohol-related harm	10.29±3.54 (0–16)	1.85±2.58 (0–14)	<0.001[a]

Notes: [a]*P*-value was calculated using *t*-test analysis. [b]*P*-value was calculated using chi-square analysis.
Abbreviations: AD, alcohol dependence; AUDIT, Alcohol Use Disorders Identification Test; F, female; M, male.

Table 2 Follow-up analysis of *BRAP* polymorphisms with the risk of alcohol dependence in Korean subjects

Loci	Allele	Position (AA change)	GWAS	MAF		Codominant			Dominant			Recessive		Statistical power (%)
			P-value	AD	Non-AD	OR (95% CI)	*P*-value[a]	P_{corr}[b]	OR (95% CI)	*P*-value[a]	P_{corr}[b]	OR (95% CI)	*P*-value[a]	
rs847895	A>G	Intron 3	–	0.336	0.300	1.22 (1.00–1.50)	**0.05**	NS	1.23 (0.94–1.60)	0.13	–	1.47 (0.94–2.31)	0.09	98.6
rs3782886	A>G	Exon 5 (R241R)	**5.0×10^{-6}**	0.041	0.159	0.19 (0.13–0.28)	**1.29×10^{-16}**	**7.74×10^{-16}**	0.19 (0.13–0.28)	**9.84×10^{-17}**	**5.90×10^{-16}**	–	–	25.2
rs3803171	G>T	Intron 5	–	0.452	0.393	1.28 (1.06–1.56)	**0.01**	NS	1.31 (0.99–1.74)	0.06	–	1.51 (1.06–2.15)	**0.02**	98.5
rs3742001	A>G	Intron 6	–	0.121	0.097	1.22 (0.89–1.67)	0.21	–	1.23 (0.89–1.71)	0.21	–	1.37 (0.25–7.58)	0.72	82.0
rs11186470	T>C	Exon 11 (R472K)	–	0.005	0.002	2.41 (0.43–13.46)	0.32	–	2.41 (0.43–13.46)	0.32	–	–	–	7.26
				Frequency										
ht1				0.502	0.444	1.29 (1.07–1.56)	**0.009**	NS	1.34 (1.00–1.81)	**0.05**	NS	1.48 (1.07–2.04)	**0.02**	97.32

Notes: Boldface indicates *P*-value <0.05. *P*-values of GWAS represent the codominant model, which includes the additive model, adjusted for age and sex as covariates. [a]*P*-value of logistic analysis under additive model by adjusting for sex and age as covariates. [b]*P*-value after Bonferroni correction for multiple testing.

Abbreviations: AA, amino acid; AD, alcohol dependence; *BRAP*, BRCA1-associated protein; GWAS, genome-wide association study; MAF, minor allele frequency; ht, haplotype.

and *rs12581829*) showed a marginal association signal with the overall AUDIT score (*P*=0.01, 0.01, and 0.008, respectively; Table S2). However, the statistical significance of these associations disappeared after corrections for multiple analyses were performed.

Discussion

AD is a distressing chronic disease that results in significant human, social, and economic burdens.[29] Drinking alcohol influences brain function by affecting brain tissues, brain cells, and the central nervous system (CNS). Accordingly, excessive alcohol consumption may result in severe deficits in cognition and memory function that are highly correlated with activity in nerve pathways.[30] In a previous GWAS from our research group,[18] *BRAP* and *PRMT8* affected neurodevelopment in brain regions that were identified as having potential susceptibility loci for AD ($P=4.65\times10^{-6}$ at *BRAP rs3782886* and $P=1.77\times10^{-5}$ at *PRMT8 rs876594*). Thus, *BRAP* and *PRMT8* were proposed as novel candidate genes for controlling the amount of alcohol consumption.

BRAP is a regulatory protein that binds to several translocation signal proteins in the cytoplasm[31] and, based on its functions, can modulate several intracellular signaling pathways. First, *BRAP* regulates the mitogen-activated protein kinase (MAPK) signaling pathway during CNS development through its function as a ubiquitin ligase.[32] MAPK signaling is a known regulator of cell survival, proliferation, and differentiation as well as the production of proinflammatory cytokines. It has also been suggested that activation of the MAPK signaling pathway contributes to the neurotropic factor-mediated regulation of alcohol consumption.[33] Second, *BRAP* acts as a primary mediator of inflammatory cascades by regulating the nuclear translocation of nuclear factor kappa B (NF-κB).[34,35] A postmortem study in humans showed that NF-κB is downregulated in the brains of alcoholic patients.[36] Similarly, other studies have shown that *BRAP* silencing via RNA interference inhibits NF-κB activation and that *BRAP* expression is ~twofold higher due to the genetic variant *rs11066001*, which is a tagging SNP of *rs3782886* that has a high correlation value ($r^2=0.81$).[31,37,38] Taken together, these findings suggest that changes in *BRAP* expression induced by genetic variants might affect the NF-κB inflammatory cascade and may be a mechanism by which *BRAP* affects the risk level of AD. However, the direct and/or indirect functional impacts of *BRAP* on AD remain to be tested because the direct functional impacts of *BRAP* on several human disorders, including schizophrenia,[31] myocardial infarction,[39] carotid atherosclerosis,[37] and metabolic syndrome,[40] are not yet fully understood. However, the function of *BRAP* as a

Table 3 Association analysis of *BRAP* polymorphisms with the AUDIT and subcategorical scores in all study subjects (n=914)

Category	Loci	C/C		C/R		R/R		*P*-value[a]	P_{corr}[b]
		N	AUDIT score	N	AUDIT score	N	AUDIT score		
AUDIT all	*rs847895*	424	17.46±11.96	399	18.57±11.91	91	20.85±10.96	**0.01**	NS
	rs3782886	736	20.36±11.12	173	9.94±11.08	5	1.20±1.64	**9.94×10^{-31}**	**5.96×10^{-30}**
	rs3803171	299	16.81±11.88	455	18.46±12.14	158	20.37±10.69	**0.002**	**0.01**
	ht1	250	16.54±11.96	463	18.43±12.20	201	20.09±10.71	**0.0007**	**0.004**
Alcohol consumption	*rs847895*	424	7.81±3.70	399	8.02±3.68	91	8.68±3.07	0.06	–
	rs3782886	736	8.73±3.15	173	5.06±3.91	5	0.40±0.55	**3.30×10^{-46}**	**1.98×10^{-45}**
	rs3803171	299	7.61±3.70	455	8.00±3.77	158	8.64±3.02	**0.005**	**0.02**
	ht1	250	7.29±3.88	463	7.98±3.73	201	8.89±2.84	**3.36×10^{-07}**	**2.02×10^{-6}**
Alcohol dependence	*rs847895*	424	3.92±4.05	399	4.36±4.13	91	4.78±3.91	**0.02**	NS
	rs3782886	736	4.74±4.06	173	2.01±3.35	5	0.00±0.00	**1.95×10^{-17}**	**1.17×10^{-16}**
	rs3803171	299	3.75±4.04	455	4.33±4.10	158	4.57±3.97	**0.03**	NS
	ht1	250	3.64±3.94	463	4.34±4.14	201	4.57±4.06	**0.009**	NS
Alcohol-related harm	*rs847895*	424	5.73±5.29	399	6.18±5.16	91	7.38±5.22	**0.007**	NS
	rs3782886	736	6.88±5.10	173	2.87±4.56	5	0.80±1.79	**3.89×10^{-22}**	**2.34×10^{-21}**
	rs3803171	299	5.45±5.16	455	6.12±5.29	158	7.16±5.07	**0.001**	**0.007**
	ht1	250	5.61±5.30	463	6.12±5.31	201	6.63±4.98	**0.03**	NS

Notes: C/C, C/R, and R/R mean major homozygote, heterozygote, and minor homozygote, respectively. AUDIT score is mean±SD. Boldface indicates *P*-value <0.05. [a]*P*-value of linear regression analysis under additive model by adjusting for sex and age as covariates. [b]*P*-value after Bonferroni correction for multiple testing.
Abbreviations: AUDIT, Alcohol Use Disorders Identification Test; BRAP, BRCA1-associated protein; ht, haplotype; NS, not significant.

mediator of the translocation of signaling proteins might be a plausible explanation for the association between *BRAP* and human diseases with distinct pathophysiologies. Taken together, these data support the notion that *BRAP* has a genetic effect on alcohol-related disorders via the control of various signaling pathways.

PRMT8 is a member of the arginine methyltransferase gene family that influences several cellular processes, such as DNA repair, RNA transcription, and signal transduction, by methylating target regions.[41] Of this protein family, only PRMT8 has an expression that is highly restricted to the CNS.[42] Several studies have reported that arginine

Table 4 Association analysis of *BRAP* polymorphisms with the AUDIT and subcategorical scores in non-alcohol dependence subjects (n=455)

Category	Loci	C/C		C/R		R/R		*P*-value[a]	P_{corr}[b]
		N	AUDIT score	N	AUDIT score	N	AUDIT score		
AUDIT all	*rs847895*	221	7.91±5.92	195	9.00±6.83	39	10.21±6.01	**0.007**	**0.04**
	rs3782886	315	10.26±6.23	135	4.91±4.88	5	1.20±1.64	**1.40×10^{-24}**	**8.39×10^{-24}**
	rs3803171	163	7.87±5.94	226	8.62±6.65	66	10.17±6.15	**0.004**	**0.02**
	ht1	138	7.44±6.35	230	8.52±6.47	87	10.53±5.65	**0.0001**	**0.0006**
Alcohol consumption	*rs847895*	221	5.57±3.43	195	5.76±3.56	39	6.49±3.05	0.11	–
	rs3782886	315	6.71±3.20	135	3.64±2.97	5	0.40±0.55	**6.46×10^{-32}**	**3.87×10^{-31}**
	rs3803171	163	5.49±3.37	226	5.67±3.58	66	6.53±3.17	**0.02**	NS
	ht1	138	4.97±3.58	230	5.61±3.42	87	7.24±2.89	**1.02×10^{-07}**	**6.13×10^{-7}**
Alcohol dependence	*rs847895*	221	0.81±1.33	195	1.14±1.62	39	1.33±1.66	**0.006**	NS
	rs3782886	315	1.22±1.59	135	0.50±1.11	5	0.00±0.00	**2.59×10^{-07}**	**1.56×10^{-6}**
	rs3803171	163	0.75±1.22	226	1.10±1.62	66	1.24±1.61	**0.004**	**0.02**
	ht1	138	0.89±1.45	230	1.06±1.60	87	0.99±1.26	0.58	–
Alcohol-related harm	*rs847895*	221	1.54±2.26	195	2.10±2.87	39	2.38±2.62	**0.008**	**0.04**
	rs3782886	315	2.33±2.76	135	0.77±1.71	5	0.80±1.79	**3.88×10^{-10}**	**2.32×10^{-9}**
	rs3803171	163	1.63±2.38	226	1.86±2.56	66	2.39±3.04	**0.03**	NS
	ht1	138	1.58±2.62	230	1.85±2.53	87	2.30±2.63	**0.04**	NS

Notes: C/C, C/R, and R/R mean major homozygote, heterozygote, and minor homozygote, respectively. AUDIT score is mean±SD. Boldface indicates *P*-value <0.05. [a]*P*-value of linear regression analysis under additive model by adjusting for sex and age as covariates. [b]*P*-value after Bonferroni correction for multiple testing.
Abbreviations: AUDIT, Alcohol Use Disorders Identification Test; BRAP, BRCA1-associated protein; ht, haplotype; NS, not significant.

methylation is important for neurogenesis, which is essential for neurologic function.[41,43] Although *PRMT8* genetic variants showed nominal association signals with the risk of AD, genetic variants of *PRMT8* might be implicated in the neuronal differentiation in the brain region.

Interestingly, the strength of the association between *BRAP* and alcohol use disorders was greater in nonalcoholic subjects than alcoholic subjects in the present study. *BRAP* is located a short distance from, and is affected by, the concomitant activity of *ALDH2*, which is highly related to AD. Thus, their association may be more prominent in nonalcoholic subjects because when *ALDH2* induces lower rates of ALDH2 catalytic activity, even a small amount of alcohol consumption can cause a dramatic enhancement in acetaldehyde levels that triggers a highly aversion reaction. Therefore, these subjects may be classified as nonalcoholics even though there is an association between *BRAP* and alcohol use disorders. *BRAP* may also be a common gene associated with the characteristic patterns of alcohol use among nonalcoholic subjects. Taken together, these findings suggest that the effects of *BRAP* in nonalcoholics are very complicated and, as a result, interpretations of the present results should be made cautiously.

Although some evidence supports an association between *BRAP* and AD, it is also important to discuss the independent effects of this gene. There are strong LD values between *BRAP rs3782886* and *ALDH2 rs671*[44] and it will be difficult to identify strong genetic influences on AD pathophysiology that arise from only a single or several genes. On the other hand, AD pathophysiology is associated with several unexplained effects from single or several genes, that is, the roles that *ADH* and *ALDH2* play in alcohol metabolism. Despite the fact that these effects are relatively small, many genes with limited effects may be involved in the pathophysiology of AD. Based on the polygenic hypothesis of AD pathophysiology, it is possible that multiple genetic loci in genes related to neurobiologic pathways could be associated with the risk of AD. Although *BRAP* has fewer independent effects in AD pathophysiology than *ALDH2*, *BRAP* may be involved in this process via the summation of many genes with small effects. The present findings suggest that *BRAP* may contribute to AD pathophysiology via contributions following the summation of its effects with the well-known effects of *ALDH2*.

Conclusion

Based on findings from a GWAS and a replication study of a Korean AD cohort, the present study was the first to propose that a *BRAP* SNP (*rs3782886*) was associated with AD. A future follow-up replication study using an independent sample may strengthen the present results and provide substantiation of the proposed polygenetic influences. Nevertheless, these novel findings provide important evidence that will contribute to the current understanding of the genetic etiology of AD as well as the development of assessments of AD risk that can be used in conjunction with conventional causal markers.

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Disclosure

Byung Lae Park is an employee and Hyung Doo Shin is the CEO of SNP Genetics, Inc., which is located at #TE1007, Teilhard Hall, Sogang University, Shinsu-dong, Mapo-gu, Seoul, 121-742, Republic of Korea. This company provided the iScan scanner instrument and BeadStudio 3.0 software used in the research. They were also involved in the study design, data collection and analysis, decision to publish, and preparation of the manuscript. However, these competing interests did not alter the authors' adherence to all policies of *Neuropsychiatric Disease and Treatment*. The others authors report no conflicts of interest in this work.

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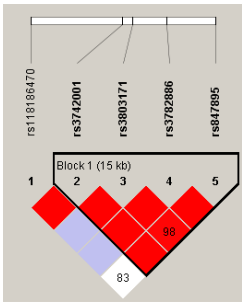
Supplementary materials

A

Hap	rs3742001	rs3803171	rs3782886	rs847895	Freq
ht1	A	G	A	A	0.471
ht2	A	T	A	G	0.319
ht3	G	T	A	A	0.107
ht4	A	G	G	A	0.100
ht5	A	G	A	G	0.003
ht6	G	G	A	A	0.0005

B



C

Block 1

Hap	rs7303822	rs16930545	rs7972007	rs7972248	Freq
ht1	T	C	T	C	0.432
ht2	T	C	T	T	0.394
ht3	G	T	C	C	0.095
ht4	T	T	C	C	0.078
ht5	G	T	C	T	0.0005
ht6	T	C	C	T	0.0005

Block 2

Hap	rs11062697	rs11062708	rs10848873	rs11062709	rs12581198	Freq
ht1	C	A	A	G	G	0.461
ht2	A	A	A	G	A	0.270
ht3	C	A	A	A	A	0.170
ht4	C	T	G	G	G	0.063
ht5	C	A	G	G	G	0.020
ht6	C	A	A	G	A	0.009
ht7	C	A	A	A	G	0.003
ht8	A	A	A	G	G	0.002
ht9	A	A	A	A	A	0.002

Block 3

Hap	rs10848876	rs3741938	Freq
ht1	C	A	0.477
ht2	T	G	0.458
ht3	T	A	0.059
ht4	C	G	0.006

Block 4

Hap	rs11062713	rs12423006	rs4766138	rs7960599	rs2159404	rs876594	rs4766139	Freq
ht1	C	C	G	A	G	G	C	0.400
ht2	C	T	A	A	A	A	T	0.215
ht3	T	T	G	A	A	A	C	0.146
ht4	T	T	G	G	A	G	C	0.106
ht5	C	T	G	A	G	G	C	0.063
ht6	C	T	G	A	A	G	C	0.060
ht7	C	T	G	A	A	A	T	0.006
ht8	C	T	G	G	A	G	C	0.002
ht9	C	T	G	A	G	G	T	0.001
ht10	C	C	G	G	G	G	C	0.001
ht11	C	T	G	A	A	A	C	0.001
ht12	T	T	G	A	A	G	C	0.001

Block 5

Hap	rs10848881	rs10774155	rs10774156	Freq
ht1	A	C	A	0.438
ht2	A	C	C	0.381
ht3	A	T	C	0.118
ht4	G	T	C	0.061
ht5	G	C	A	0.002

Block 6

Hap	rs11614792	rs9669266	rs12581829	Freq
ht1	C	A	A	0.497
ht2	T	G	A	0.258
ht3	T	A	A	0.122
ht4	T	G	G	0.119
ht5	C	G	G	0.004

Block 7

Hap	rs10774158	rs4766141	rs3782749	rs11062723	Freq
ht1	G	C	A	A	0.397
ht2	G	C	A	G	0.225
ht3	G	G	A	G	0.197
ht4	A	C	A	G	0.121
ht5	A	C	G	G	0.059
ht6	G	G	A	A	0.001

Block 8

Hap	rs3741936	rs917602	Freq
ht1	C	C	0.462
ht2	T	C	0.277
ht3	C	T	0.256
ht4	T	T	0.004

Block 9

Hap	rs7966000	rs7957814	rs6489480	Freq
ht1	A	G	A	0.853
ht2	G	A	G	0.111
ht3	G	G	A	0.034
ht4	G	G	G	0.001
ht5	A	A	A	0.001

Block 10

Hap	rs7307502	rs3782744	rs7976970	rs16930578	rs758637	rs7137875	rs11062731	Freq
ht1	A	T	A	G	C	A	G	0.470
ht2	A	C	A	G	T	A	G	0.350
ht3	G	C	G	A	C	G	C	0.080
ht4	G	C	A	G	T	A	G	0.034
ht5	G	C	G	G	C	G	C	0.032
ht6	G	C	A	G	C	G	C	0.020
ht7	G	C	A	G	C	G	G	0.008
ht8	A	C	A	G	C	A	G	0.003
ht9	G	C	G	G	C	G	G	0.002
ht10	A	C	G	G	C	G	C	0.001

Block 11

Hap	rs11062733	rs4765741	rs11830814	rs2159347	rs1029766	Freq
ht1	C	A	G	G	C	0.468
ht2	C	G	G	G	T	0.304
ht3	C	G	G	A	T	0.102
ht4	T	G	C	G	C	0.080
ht5	T	G	G	G	C	0.022
ht6	C	G	G	G	C	0.020
ht7	C	G	G	A	C	0.003
ht8	C	A	C	G	C	0.001
ht9	C	A	G	G	T	0.001

Figure S1 (*Continued*)

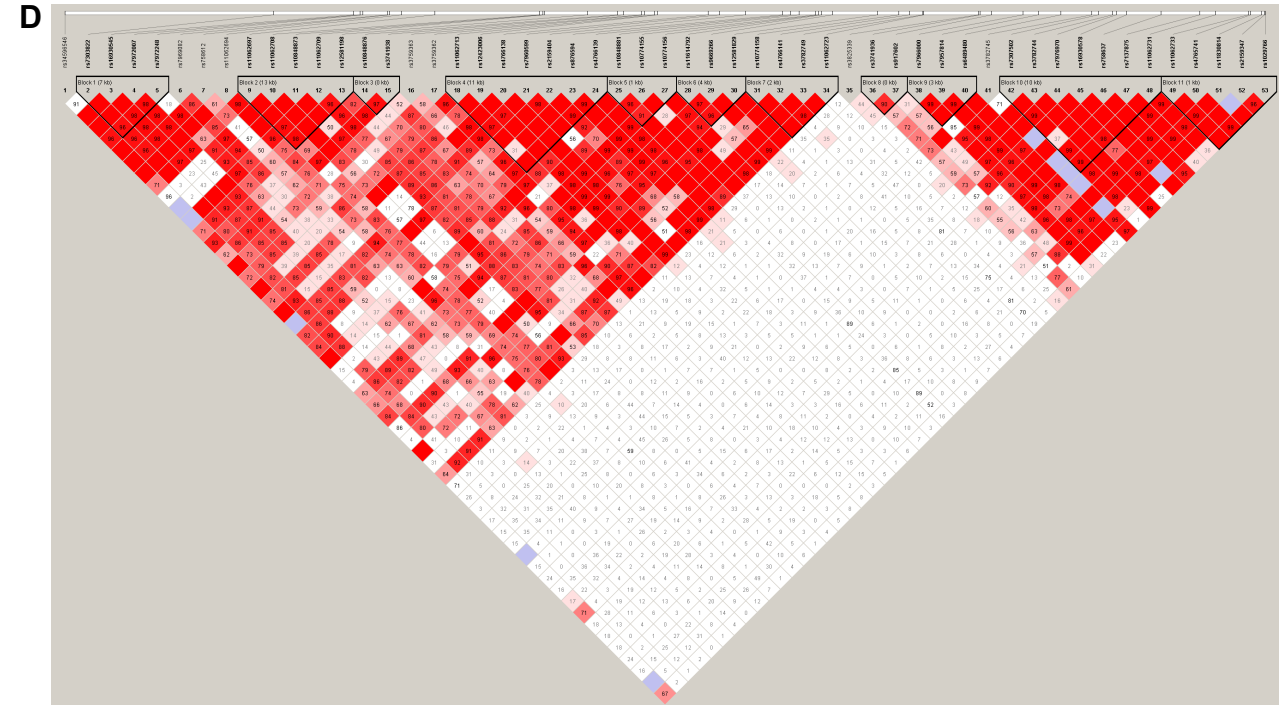


Figure S1 Haplotypes and LD structures of *BRAP* and *PRMT8*.
Notes: (**A**) Haplotypes of and (**B**) LDs among *BRAP* polymorphisms. (**C**) Haplotypes of and (**D**) LDs among *PRMT8* polymorphisms.
Abbreviations: BRAP, BRCA1-associated protein; LD, linkage disequilibrium; PRMT8, protein arginine methyltransferase.

Table S1 Association analysis between *PRMT8* SNPS and alcohol dependence subjects (n=914)

Loci	Allele	Position	GWAS *P*-value	MAF		Codominant		Dominant		Recessive		Statistical power (%)
				AL	Non-AL	OR (95% CI)	*P*-value*	OR (95% CI)	*P*-value*	OR (95% CI)	*P*-value*	
rs34596546	T>C	Intron	–	0.038	0.032	1.17 (0.70–1.97)	0.55	1.17 (0.70–1.97)	0.55	–	–	43.6
rs7303822	T>G	Intron	–	0.090	0.092	0.96 (0.69–1.33)	0.80	0.93 (0.66–1.32)	0.68	1.51 (0.35–6.52)	0.58	78.8
rs16930545	C>T	Intron	–	0.170	0.166	1.05 (0.81–1.35)	0.72	1.08 (0.81–1.43)	0.62	0.90 (0.38–2.12)	0.80	94.3
rs7972007	T>C	Intron	–	0.172	0.166	1.07 (0.83–1.38)	0.61	1.10 (0.83–1.46)	0.51	0.89 (0.38–2.11)	0.79	94.4
rs7972248	C>T	Intron	**0.02**	0.388	0.407	0.93 (0.76–1.13)	0.45	0.90 (0.69–1.19)	0.48	0.91 (0.62–1.34)	0.64	99.1
rs7969082	G>A	Intron	0.53	0.252	0.265	0.98 (0.79–1.21)	0.84	0.95 (0.73–1.24)	0.71	1.06 (0.63–1.79)	0.83	97.9
rs758612	A>C	Intron	0.61	0.200	0.187	1.13 (0.88–1.43)	0.34	1.14 (0.86–1.50)	0.37	1.23 (0.58–2.61)	0.58	95.9
rs11062694	T>C	Intron	**0.03**	0.191	0.193	0.96 (0.75–1.21)	0.70	0.93 (0.71–1.23)	0.61	1.06 (0.52–2.13)	0.88	96.0
rs11062697	C>A	Intron	0.10	0.283	0.267	1.06 (0.86–1.30)	0.58	1.08 (0.82–1.40)	0.59	1.08 (0.67–1.74)	0.74	98.3
rs11062708	A>C	Intron	–	0.060	0.068	0.95 (0.64–1.40)	0.78	1.00 (0.67–1.50)	1	–	–	61.6
rs10848873	A>C	Intron	0.53	0.075	0.093	0.82 (0.58–1.17)	0.28	0.85 (0.60–1.22)	0.39	–	–	68.3
rs11062709	G>A	Intron	–	0.176	0.178	0.98 (0.77–1.24)	0.84	0.97 (0.73–1.29)	0.86	0.95 (0.46–1.96)	0.89	95.0
rs12581198	G>A	Intron	–	0.459	0.447	1.02 (0.85–1.23)	0.83	1.06 (0.79–1.42)	0.69	0.99 (0.71–1.37)	0.94	99.2
rs10848876	T>C	Intron	–	0.480	0.493	0.94 (0.77–1.13)	0.50	1.12 (0.83–1.52)	0.46	0.74 (0.54–1.01)	0.06	99.2
rs3741938	T>C	Intron	**0.0006**	0.461	0.462	1.01 (0.83–1.22)	0.95	1.14 (0.85–1.53)	0.38	0.86 (0.62–1.20)	0.38	99.3
rs3759363	A>G	Intron	**0.006**	0.212	0.189	1.18 (0.93–1.51)	0.18	1.20 (0.91–1.59)	0.19	1.24 (0.57–2.70)	0.58	96.0
rs3759362	T>C	Intron	**0.0005**	0.466	0.469	1.00 (0.82–1.21)	0.97	1.06 (0.79–1.43)	0.70	0.92 (0.67–1.28)	0.63	99.3
rs11062713	C>T	Intron	–	0.266	0.240	1.18 (0.94–1.47)	0.15	1.19 (0.91–1.56)	0.20	1.35 (0.76–2.40)	0.31	97.7
rs12423006	T>C	Intron	–	0.406	0.400	1.01 (0.83–1.24)	0.89	1.11 (0.84–1.47)	0.47	0.88 (0.60–1.27)	0.48	99.3
rs4766138	G>A	Intron	**0.02**	0.198	0.226	0.84 (0.67–1.06)	0.14	0.96 (0.73–1.26)	0.74	0.33 (0.17–0.66)	**0.002**	95.2
rs7960599	A>G	Intron	–	0.108	0.102	1.09 (0.80–1.48)	0.59	1.03 (0.74–1.44)	0.84	3.64 (0.70–18.83)	0.12	83.8
rs2159404	T>C	Intron	–	0.473	0.460	1.03 (0.85–1.25)	0.76	1.04 (0.77–1.39)	0.81	1.04 (0.76–1.44)	0.79	99.2
rs876594	C>T	Intron	**0.00002**	0.362	0.373	0.96 (0.78–1.16)	0.64	1.03 (0.78–1.35)	0.85	0.79 (0.53–1.17)	0.23	99.1
rs4766139	C>T	Intron	**0.03**	0.205	0.234	0.84 (0.67–1.05)	0.13	0.98 (0.74–1.28)	0.86	0.30 (0.15–0.59)	**0.0005**	95.7
rs10848881	A>C	Intron	–	0.059	0.065	0.96 (0.65–1.41)	0.82	0.97 (0.64–1.45)	0.86	0.64 (0.06–7.10)	0.71	61.5
rs10774155	C>T	Intron	–	0.179	0.177	1.04 (0.81–1.33)	0.77	1.15 (0.87–1.52)	0.34	0.50 (0.22–1.13)	0.10	95.2
rs10774156	C>A	Intron	**0.005**	0.451	0.434	1.05 (0.87–1.28)	0.60	1.13 (0.84–1.50)	0.42	1.00 (0.71–1.40)	0.99	99.2
rs11614792	T>C	Intron	–	0.492	0.501	0.96 (0.80–1.17)	0.71	1.07 (0.79–1.46)	0.66	0.85 (0.62–1.16)	0.29	99.2
rs9669266	A>G	Intron	–	0.369	0.390	0.91 (0.75–1.10)	0.33	0.98 (0.75–1.29)	0.91	0.71 (0.48–1.04)	0.08	99.0
rs12581829	A>C	Intron	–	0.105	0.137	0.72 (0.54–0.96)	**0.02**	0.71 (0.51–0.97)	**0.03**	0.52 (0.20–1.36)	0.18	79.1
rs10774158	G>A	Intron	0.09	0.179	0.179	1.02 (0.80–1.31)	0.85	1.14 (0.86–1.52)	0.36	0.46 (0.20–1.04)	0.06	95.3
rs4766141	C>G	Intron	–	0.210	0.187	1.16 (0.91–1.47)	0.24	1.16 (0.88–1.53)	0.30	1.39 (0.68–2.87)	0.37	95.9
rs3782749	T>G	Intron	–	0.054	0.064	0.90 (0.60–1.34)	0.60	0.90 (0.59–1.37)	0.63	0.64 (0.06–7.08)	0.71	56.6
rs11062723	G>A	Intron	–	0.409	0.393	1.07 (0.88–1.30)	0.52	1.12 (0.85–1.48)	0.41	1.03 (0.71–1.48)	0.90	99.2
rs3825339	A>G	Intron	–	0.224	0.210	1.10 (0.88–1.39)	0.40	1.13 (0.86–1.48)	0.40	1.12 (0.59–2.12)	0.74	96.9
rs3741936	G>A	Intron	0.14	0.272	0.285	0.93 (0.75–1.15)	0.49	0.92 (0.70–1.20)	0.52	0.90 (0.53–1.53)	0.69	98.3
rs917602	G>A	Intron	0.28	0.264	0.253	1.05 (0.85–1.31)	0.63	1.11 (0.85–1.45)	0.44	0.92 (0.55–1.55)	0.75	98.1
rs7966000	A>G	Intron	–	0.136	0.156	0.82 (0.63–1.08)	0.16	0.81 (0.60–1.09)	0.16	0.76 (0.28–2.11)	0.60	88.8

rs7957814	G>A	Intron	0.15	0.111	0.113	0.98 (0.73–1.32)	0.89	0.97 (0.70–1.34)	0.85	1.14 (0.30–4.36)	0.84	85.5
rs6489480	A>G	Intron	–	0.111	0.114	0.97 (0.72–1.31)	0.84	0.96 (0.69–1.32)	0.79	1.14 (0.30–4.36)	0.84	85.2
rs3782745	A>C	Intron	–	0.053	0.045	1.17 (0.76–1.79)	0.48	1.10 (0.70–1.72)	0.68	–	–	55.7
rs7307502	A>G	Intron	–	0.176	0.176	0.97 (0.76–1.24)	0.82	0.94 (0.70–1.24)	0.65	1.23 (0.57–2.65)	0.61	95.1
rs3782744	G>A	Intron	0.44	0.479	0.464	1.09 (0.91–1.32)	0.34	0.98 (0.74–1.32)	0.91	1.33 (0.97–1.82)	0.08	99.2
rs7976970	A>G	Intron	0.14	0.115	0.115	1.00 (0.74–1.35)	0.99	0.99 (0.72–1.37)	0.97	1.14 (0.30–4.36)	0.84	86.7
rs16930578	G>A	Intron	0.11	0.083	0.077	1.07 (0.75–1.52)	0.72	1.10 (0.76–1.58)	0.62	0.42 (0.04–4.69)	0.48	74.7
rs758637	G>A	Intron	0.93	0.381	0.382	0.98 (0.81–1.18)	0.80	0.89 (0.68–1.17)	0.41	1.13 (0.78–1.63)	0.53	99.2
rs7137875	A>G	Intron	0.34	0.137	0.149	0.89 (0.68–1.16)	0.38	0.88 (0.66–1.19)	0.41	0.76 (0.28–2.11)	0.60	89.7
rs11062731	G>C	Intron	–	0.129	0.138	0.90 (0.68–1.20)	0.47	0.90 (0.66–1.22)	0.50	0.81 (0.27–2.50)	0.72	88.7
rs11062733	C>T	Intron	0.22	0.099	0.102	0.94 (0.68–1.29)	0.69	0.94 (0.68–1.32)	0.73	0.69 (0.11–4.31)	0.69	81.7
rs4765741	G>A	Intron	0.47	0.479	0.466	1.08 (0.90–1.30)	0.42	0.99 (0.74–1.32)	0.92	1.27 (0.93–1.74)	0.14	99.2
rs11830814	G>C	Intergenic	–	0.082	0.078	1.06 (0.74–1.50)	0.76	1.08 (0.75–1.56)	0.67	0.42 (0.04–4.69)	0.48	74.8
rs2159347	C>T	Intergenic	–	0.117	0.091	1.31 (0.96–1.78)	0.09	1.35 (0.97–1.88)	0.08	1.21 (0.31–4.65)	0.79	82.5
rs1029766	G>A	Intergenic	0.77	0.402	0.407	0.97 (0.80–1.17)	0.72	0.86 (0.65–1.13)	0.28	1.14 (0.80–1.62)	0.47	99.3
				Frequency								
BL1_ht1				0.441	0.429	1.03 (0.85–1.25)	0.74	1.10 (0.82–1.46)	0.53	0.97 (0.69–1.37)	0.88	99.1
BL1_ht4				0.081	0.074	1.19 (0.82–1.72)	0.35	1.19 (0.82–1.72)	0.35	–	–	71.3
BL2_ht1				0.461	0.455	1.03 (0.85–1.25)	0.76	1.13 (0.85–1.52)	0.40	0.93 (0.67–1.30)	0.69	99.2
BL3_ht3				0.062	0.055	1.12 (0.75–1.66)	0.59	1.19 (0.78–1.82)	0.41	0.27 (0.03–2.68)	0.27	59.8
BL4_ht3				0.158	0.138	1.19 (0.91–1.56)	0.20	1.16 (0.86–1.56)	0.32	2.14 (0.72–6.34)	0.17	89.3
BL4_ht5				0.065	0.060	1.04 (0.71–1.54)	0.84	1.09 (0.72–1.63)	0.69	0.29 (0.03–3.29)	0.32	63.4
BL4_ht6				0.056	0.064	0.92 (0.62–1.38)	0.70	0.96 (0.63–1.44)	0.83	–	–	56.3
BL5_ht2				0.370	0.389	0.93 (0.76–1.13)	0.44	1.01 (0.77–1.33)	0.94	0.73 (0.49–1.07)	0.10	98.8
BL5_ht3				0.121	0.112	1.09 (0.81–1.47)	0.57	1.09 (0.80–1.50)	0.59	1.21 (0.33–4.40)	0.77	85.1
BL6_ht2				0.265	0.253	1.08 (0.87–1.35)	0.48	1.09 (0.84–1.43)	0.51	1.13 (0.62–2.05)	0.69	97.8
BL6_ht3				0.125	0.116	1.09 (0.82–1.46)	0.55	1.11 (0.81–1.53)	0.50	0.94 (0.27–3.23)	0.92	86.1
BL7_ht2				0.205	0.241	0.81 (0.65–1.02)	0.07	0.94 (0.72–1.24)	0.68	0.28 (0.14–0.55)	**0.0002**	93.6
BL7_ht4				0.123	0.115	1.08 (0.81–1.45)	0.60	1.10 (0.80–1.51)	0.55	0.94 (0.27–3.23)	0.92	85.9
BL8_ht1				0.467	0.466	1.02 (0.85–1.22)	0.85	0.91 (0.68–1.21)	0.51	1.18 (0.86–1.61)	0.31	99.3
BL10_ht2				0.341	0.356	0.92 (0.76–1.12)	0.41	0.83 (0.63–1.08)	0.16	1.09 (0.73–1.64)	0.68	98.8
BL11_ht2				0.288	0.319	0.85 (0.69–1.04)	0.12	0.82 (0.63–1.06)	0.13	0.81 (0.51–1.30)	0.38	97.5

Notes: Boldface indicates *P*-value <0.05. *P*-values of GWAS represent the codominant model, which includes the additive model, adjusted for age and sex as covariates. **P*-value of logistic analysis under additive model by adjusting for sex and age as covariates.

Abbreviations: AA, amino acid; AL, alcoholic subjects; GWAS, genome-wide association study; ht, haplotype; MAF, minor allele frequency; PRMT8, protein arginine methyltransferase.

Table S2 Association analysis of *PRMT8* SNPs with the AUDIT score in all study subjects (n=914)

Category	Loci	C/C		C/R		R/R		*P*-value*
		N	AUDIT score	N	AUDIT score	N	AUDIT score	
AUDIT all	*rs4766138*	572	18.77±11.79	296	18.20±11.81	46	12.65±12.10	**0.01**
	rs4766139	562	18.75±11.79	303	18.36±11.82	49	12.33±11.80	**0.01**
	rs12581829	711	18.81±11.76	183	16.34±12.01	19	16.21±13.06	**0.008**
Alcohol consumption	*rs4766138*	572	8.12±3.58	296	7.98±3.61	46	6.48±4.25	**0.04**
	rs4766139	562	8.12±3.58	303	8.01±3.61	49	6.43±4.13	**0.03**
	rs12581829	711	8.13±3.56	183	7.45±3.88	19	7.74±3.86	**0.03**
Alcohol dependence	*rs4766138*	572	4.31±4.10	296	4.21±4.02	46	2.74±3.87	0.08
	rs4766139	562	4.28±4.09	303	4.30±4.05	49	2.59±3.80	0.09
	rs12581829	711	4.30±4.08	183	3.83±4.03	19	3.53±4.36	0.09
Alcohol-related harm	*rs4766138*	572	6.34±5.22	296	6.02±5.21	46	3.43±4.96	**0.005**
	rs4766139	562	6.35±5.23	303	6.06±5.20	49	3.31±4.86	**0.003**
	rs12581829	711	6.38±5.23	183	5.07±5.11	19	4.95±5.71	**0.001**

Notes: C/C, C/R, and R/R mean major homozygote, heterozygote, and minor homozygote, respectively. AUDIT score is mean±SD. *P-value of linear regression analysis under additive model by adjusting for sex and age as covariates. Boldface indicates *P*-value <0.05.
Abbreviations: AUDIT, Alcohol Use Disorders Identification Test; PRMT8, protein arginine methyltransferase.