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Bivariate genome-wide association analyses identified genetic pleiotropic effects for bone mineral density and alcohol drinking in Caucasians

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Abstract

Several studies indicated bone mineral density (BMD) and alcohol intake might share common genetic factors. The study aimed to explore potential SNPs/genes related to both phenotypes in US Caucasians at the genome-wide level. A bivariate genome-wide association study (GWAS) was performed in 2069 unrelated participants. Regular drinking was graded as 1, 2, 3, 4, 5, or 6, representing drinking alcohol never, less than once, once or twice, three to six times, seven to ten times, or more than ten times per week respectively. Hip, spine, and whole body BMDs were measured. The bivariate GWAS was conducted on the basis of a bivariate linear regression model. Sex-stratified association analyses were performed in the male and female subgroups. In males, the most significant association signal was detected in SNP rs685395 in *DYNC2H1* with bivariate spine BMD and alcohol drinking ($P = 1.94 \times 10^{-8}$). SNP rs685395 and five other SNPs, rs657752, rs614902, rs682851, rs626330, and rs689295, located in the same haplotype block in *DYNC2H1* were the top ten most significant SNPs in the bivariate GWAS in males. Additionally, two SNPs in *GRIK4* in males and three SNPs in *OPRM1* in females were suggestively associated with BMDs (of the hip, spine, and whole body) and alcohol drinking. Nine SNPs in *ILIRN* were only suggestively associated with female whole body BMD and alcohol drinking. Our study indicated that *DYNC2H1* may contribute to the genetic mechanisms of both spine BMD and alcohol drinking in male Caucasians. Moreover, our study suggested potential pleiotropic roles of *OPRM1*

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

and *IL1RN* in females and *GRIK4* in males underlying variation of both BMD and alcohol drinking.

Keywords

Bone mineral density; Alcohol drinking; Bivariate genome-wide association study; *DYNC2H1*

Introduction

Osteoporosis is the most common and most serious skeletal disorder among the elderly. Osteoporosis is characterized by a low bone mineral density (BMD), with a strong genetic component; heritability is estimated to be as high as 85% [1]. In recent years, several genes/loci affecting BMD have been identified by a variety of experimental approaches [2, 3]. As epidemiologic studies of osteoporosis continue, especially in countries with aging populations, researchers have begun to focus on the effects of lifestyle and habitual social behavior on osteoporosis, and to try to explore the common genetic factors between BMD and the phenotype concerned. One particular social behavior that has attracted a great deal of attention for its potential impact on osteoporosis is alcohol consumption [4].

Worldwide, about two billion people are alcohol consumers, with 76.3 million having diagnosable alcohol use disorders [5]. Animal and human studies indicate strain, species, and racial differences in biological responses to alcohol [6]. Twin registry and adoption studies have shown that the heritability of alcoholism may be as high as 50–60% [7]. A meta-analysis of 9382 participants in 42 case–control studies and two cross-sectional surveys implicated *DRD2* Tag1A polymorphism at 11q23 as having a small but significant association with the risk of alcohol dependence [8]. Recently, through the study of the subjective effects of alcohol on individuals categorized as having intermediate phenotype alcohol disorders, several risk genes have been identified (such as genes encoding the classic opoid receptor, *OPRM1*, the major inhibitory neurotransmitter in the central nervous system, *GABA*, and alcohol metabolizing enzymes, *ALDH2*) [9–11]. Some analyses of alcohol dependence genome-wide association studies (GWAS) and post-GWAS analyses are constantly emerging that seek to identify loci associated with alcohol dependence [11].

An increasing number of studies have demonstrated the correlation between BMD and alcohol drinking in both men and women [12, 13]. Alcoholism is a risk factor for osteoporotic fractures and low BMD [4]. Human, animal, and cell culture studies of the correlation between alcohol consumption and BMD strongly suggest that alcohol has a dose-dependent toxic effect on osteoblast activity. Although a large number of studies have reported that moderate intake of alcohol appears to increase BMD in men and perimenopausal women [14, 15], long-term consumption of excessive amounts of alcohol eventually results in osteopenia, increased risk of osteoporosis, and delayed healing of fractures.

From the genetic perspective, alcohol consumption and BMD might have common genetic mechanisms. Some epidemiologic observations suggest that alcohol-preference quantitative trait loci in preferring/nonpreferring rats also harbor a gene or genes that affect bone

structure [16]. The mechanisms of action of alcohol on bone are thought to be both direct by changing the number and activity of the osteoblasts and osteoclasts and indirect via a decrease of calorie intake and a change of body composition [4].

Although univariate GWAS results show that both BMD and alcohol drinking are significantly influenced by genetic factors with heritability exceeding 50%, the question is whether the comorbidity results from the same genes influencing both traits. In view of complex disease/trait, univariate analysis is theoretically less powerful for the potential genetic correlation between correlated traits, so it is difficult to identify the pleiotropic genes (common genes regulating several traits) those with a set of related diseases/traits that play an important role in the pathogenesis [17]. Pei et al. [18] developed a bivariate analysis of two correlated quantitative traits based on a haplotype trend regression. This bivariate model is used to test the association between two continuous phenotypes in unrelated individuals genotyped at one SNP marker. There is no report of covariants of BMD and alcohol drinking, so the purpose of the current study was to identify common SNPs/genes that contribute to both phenotypes of BMD and alcohol drinking by means of a bivariate GWAS in Caucasians.

Materials and methods

Ethics statement

The study was approved by institutional review boards of Creighton University and the University of Missouri-Kansas City. Signed informed consent documents were obtained from all participants before they were enrolled in the study.

Participants

The test sample was an osteoporosis study sample consisting of 2069 unrelated US Caucasians (509 males, 1560 females) recruited in the Midwestern USA (Kansas City, MO, and Omaha, NE). Strict exclusion criteria were adopted to minimize any known and potential confounding factors influencing variation of BMD. Individuals with chronic disorders involving vital organs (heart, lung, liver, kidney and brain), serious metabolic diseases (diabetes, hypoparathyroidism, hyperparathyroidism, hyperthyroidism, etc.), skeletal diseases (Paget disease, osteogenesis imperfecta, rheumatoid arthritis, etc.), chronic use of drugs affecting bone metabolism (hormone replacement therapy, corticosteroid therapy, anticonvulsant drugs), and malnutrition conditions (chronic diarrhea, chronic ulcerative colitis, etc.) were excluded from the present study.

Phenotyping

In Diagnostic Interview for Genetic Studies (DIGS) [19], “regular drinking” is defined as drinking at least once a week. For all participants, alcohol drinking behavior information was collected. Alcohol drinking was graded as 1, 2, 3, 4, 5, or 6, representing drinking alcohol never, less than once, once or twice, three to six times, seven to ten times, or more than ten times per week respectively. Hip BMD, spine BMD, and whole body BMD (g/cm^2) were measured by dual-energy X-ray absorptiometry with a Hologic QDR-4500 W instrument in the 2069 participants.

Genotyping

Genomic DNA was extracted from peripheral blood leukocytes with the Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN, USA) for each study participant. All participants were individually genotyped with the Affymetrix human mapping SNP 6.0 assay according to the manufacturer's recommendation. For quality control of SNPs, we set the default value of greater than 0.4 as the contrast quality control threshold. Samples with a minimum call rate of 95% were included in the analyses. For the Caucasian sample, the final mean call rate reached a level of 98.93%. We discarded SNPs that deviated from Hardy–Weinberg equilibrium ($P < 0.0001$) and those with a minor allele frequency less than 0.01. After quality control, 69,108 SNPs were retained for subsequent analyses.

Statistical analyses

Before phenotype–genotype association analyses, we first estimated the phenotypic correlation between BMD and alcohol drinking in our 2069 Caucasian participants with SPSS.

Before association analyses, both raw phenotypes of alcohol drinking and BMD phenotypes at three sites (hip, spine, and whole body) were adjusted by height, weight, age, and age squared by stepwise regression with SPSS. To correct for the effect of potential population stratification, we performed a principal component analysis on genome-wide SNP data with EIGENSTRAT and included the top ten principal components as covariates. The covariate-adjusted phenotypic values (z score) were used in subsequent association analyses. To ensure adequate normality of quantitative traits, we removed the outliers of both adjusted phenotypes on the basis of an inverse normal transformation.

We performed bivariate GWAS to detect associations between each SNP and two phenotypes of BMD and alcohol drinking. Sex-stratified association analyses were performed in the male and female subgroups. Haplotype association analyses of interesting SNP groups were performed with bivariate frameworks. An additive genetic model and a linear regression model were applied to both univariate and bivariate association analyses with use of the R package [18]. This method is expressed as the formula

$$\begin{pmatrix} y_{i1} \\ y_{i2} \end{pmatrix} = \begin{pmatrix} \mu_1 \\ \mu_2 \end{pmatrix} + (Z_1 Z_2 \dots Z_n) \begin{pmatrix} \beta_{11}^{(0)} & \beta_{12}^{(0)} \\ \beta_{21}^{(0)} & \beta_{22}^{(0)} \\ \dots & \dots \\ \beta_{n1}^{(0)} & \beta_{n2}^{(0)} \end{pmatrix} + \begin{pmatrix} \beta_1 \\ \beta_2 \end{pmatrix} x_i + \begin{pmatrix} \varepsilon_{i1} \\ \varepsilon_{i2} \end{pmatrix}$$

where for an individual i , y_i is a vector with a length of 1 (for univariate analysis) or 2 (for

bivariate analysis) coding the individual's phenotype, $\mu = \begin{pmatrix} \mu_1 \\ \mu_2 \end{pmatrix}$ is the grand mean vector, $Z = (Z_1 Z_2 \dots Z_n)$ is a vector coding for covariates, which may include other risk factors and confounding factors, β is the corresponding effects of covariates or the SNP being tested, x_i is the genotype score at the locus of interest for individual i , and ε_i is the vector of random error. We compared the likelihood of the model under the null hypothesis ($\beta = 0$) with that under the alternative hypothesis ($\beta \neq 0$), to test the alternative hypothesis. Then the

likelihood ratio can be converted to an F statistic, which follows an F distribution under the null hypothesis. The P value was calculated on the basis of the F statistic. The linkage disequilibrium [standardized D' ($D/D_{\max}$)] patterns of interesting SNPs and the haplotype block map were analyzed with Haploview (<http://www.broad.mit.edu/mpg/haploview/>). Phased haplotypes of each block were obtained with PLINK (version 1.07, <http://pngu.mgh.harvard.edu/~purcell/plink/>) in this bivariate GWAS. We also performed univariate GWAS with each of the tested SNPs to compare the results between bivariate and univariate analysis.

We estimated the statistical power of bivariate association analyses of BMD and alcohol drinking, and univariate association analyses of each trait in 506 males and 1553 females, separately. We performed power analyses using the R package *GEE* (generalized estimation equation) (<http://cran.r-project.org/src/contrib/Descriptions/geepack.html>) for genotype-based bivariate association analyses under the additive genetic models. We used ANOVA in R and performed power analyses of genotype-based univariate association analyses. Five thousand replicates were run in the simulation to calculate the power.

Results

The basic characteristics of the study participants are shown in Table 1. BMDs at all study sites (hip, spine, and whole body) were lower in females than in males. The ratios of drinkers were nearly equal in females (74.5%) and males (75.4%).

Correlation analysis of our phenotype data showed that alcohol drinking was significantly correlated with hip and spine BMD in males, with correlation coefficients of 0.677 and 0.680 ($P < 0.001$) for alcohol drinking–hip BMD and alcohol drinking–spine BMD respectively. The correlation coefficients were 0.114, 0.100, and 0.078 for alcohol drinking–hip BMD, alcohol drinking–spine BMD, and alcohol drinking–whole body BMD in females and 0.009 for alcohol drinking–whole body BMD in males.

Of the bivariate GWAS results, we concentrated on SNPs with $P < 10^{-4}$. Of these SNPs, we initially focused on SNPs located in genes that have previously been associated with either BMD or alcohol drinking, or both. Next, we focused on interesting SNPs/genes that had not previously been associated with either BMD or alcohol drinking and that appeared to function as pleiotropic loci for both BMD and alcohol drinking in the current sample of Caucasians.

Figure 1 shows the distribution of P values of bivariate GWAS across the genome in the Caucasian sample. In males, we identified seven interesting SNPs in the *DYNC2H1* gene (which encodes dynein cytoplasmic 2 heavy chain 1) that were bivariately associated with spine BMD and alcohol drinking (Table 2). The most promising SNP was rs685395 (T/C) ($P = 1.94 \times 10^{-8}$), located in an intron of *DYNC2H1* at chromosome locus 11q22.3; six other SNPs in *DYNC2H1* in the same haplotype block ($D' > 0.99$) yielded suggestive associations, with P values ranging from 3.28×10^{-5} to 8.57×10^{-7} . Among the seven SNPs, six SNPs (rs685395, rs657752, rs614902, rs682851, rs626330, rs689295) ranked among the top ten most significant SNPs in the present study with bivariate $P < 10^{-5}$.

Additionally, SNPs rs7103821 and rs12284644 in the *GRIK4* gene (which encodes glutamate ionotropic receptor kainate type subunit 4) were suggestively associated with all study BMD phenotypes (hip, spine, and whole body) and alcohol drinking in males (Table 2). In females, three SNPs in the *OPRM1* gene (which encodes μ -opioid receptor 1)—rs9479769, rs4870268, and rs1998221—were suggestively associated with all BMD phenotypes (hip, spine, and whole body) and alcohol drinking. Nine additional SNPs (rs444413, rs432014, rs423904, rs419598, rs442710, rs447713, rs451578, rs431726, rs4251984) located in the *IL1RN* gene (which encodes IL-1 receptor antagonist) were suggestively associated with whole body BMD and alcohol drinking in females (Table 3).

As shown in Tables 2 and 3, bivariate association signals were generally stronger than univariate association signals. The results of univariate analyses showed that seven SNPs in *DYNC2H1* were nominally associated with spine BMD in the univariate analyses in males ($P < 10^{-3}$). Additionally, three SNPs in *OPRM1* were nominally associated with alcohol drinking in females ($P < 10^{-2}$).

Haplotype analyses constructed by the SNP groups located in *DYNC2H1* and *GRIK4* in males and the SNP groups located in *IL1RN* and *OPRM1* in females are shown in Fig. 2. In males, the seven SNPs in *DYNC2H1* were in one haplotype block, and the two SNPs in *GRIK4* were in another block on chromosome 11. In females, the nine SNPs in *IL1RN* were in tight linkage disequilibrium in one haplotype block and the three SNPs in *OPRM1* were in another block.

Discussion

To our knowledge, this is the first bivariate GWAS for BMD and alcohol drinking, and suggests several genes (*DYNC2H1*, *GRIK4*, *IL1RN*, *OPRM1*) may contribute to covariation of BMD and alcohol drinking. Although univariate analysis has been providing valuable insights into the genetics of common and complex diseases, bivariate GWAS considers the correlation between traits and has the advantage of identifying pleiotropic loci that can coregulate two related traits.

In the present bivariate GWAS, the findings strongly suggest that, in males, *DYNC2H1* is a potential candidate genetic variant for both spine BMD and alcohol drinking. From the perspective of biological processes, it has been reported that mutations in *DYNC2H1* can lead to chondrodysplasia, and that this gene was essential for skeletogenesis and growth [20, 21]. Also, mutations in human *DYNC2H1* are associated with short rib–polydactyly syndrome type III and asphyxiating thoracic dystrophy [21, 22]. These two related skeletal dysplasias are characterized by shortened long bones, a narrow rib cage and polydactyly, and other features of ciliopathies. Chondrocyte cilia from individuals with *DYNC2H1* mutations are shortened with bulbous distal ends [22]. In particular, a number of studies have suggested that there is a connection between cilia and Wnt signaling [23]. A systematic analysis of canonical Wnt signaling in mutant mouse embryos with abnormal primary cilia due to defects in *DYNC2H1* showed that *DYNC2H1* may play an indirect role in Wnt signaling [24], which is recognized as a classic signaling pathway related to bone development. However, the potential mechanisms by which *DYNC2H1* variants influence

alcohol drinking are completely unknown. A GWAS has identified groups of genomic markers, including *DYNC2H1*, associated with successful quitting in a smoking cessation trial that the examined effects of precessation nicotine replacement therapy [25]. Homozygous missense mutations in *DYNC2H1* reduce the rate of retrograde transport in motor neurons and affect the survival of sensory neurons [26]. These observations indicate that defects in axonal transport are associated with neuronal degeneration. Three genetic pathways were associated with *DYNC2H1*: vasopressin-regulated water reabsorption, phagosome function, and *Salmonella* infection. Among them, the vasopressin-regulated water reabsorption and phagosome function pathways mainly reflect intracellular processes, transport, and catabolism. Both of these pathways have been associated with asphyxiating thoracic dystrophy caused by shortened ribs and the nervous system disease distal hereditary motor neuropathies. The analyses conducted in the current study provide support for the concept that *DYNC2H1* is a potential candidate gene affecting both spine BMD and alcohol drinking in males.

The current study also provided potential pleiotropic roles of *GRIK4* in males and *OPRM1* and *IL1RN* in females affecting BMD and alcohol drinking. *GRIK4* is a critical regulator of network activity that acts by modifying neuronal excitability through GABA (gamma amino acid butyric acid)ergic interneurons [27]. A GWAS in 367 Caucasian participants found *GRIK4* as a leading edge gene contributing to human variants in alcohol response [28]. The effect of *GRIK4* on alcohol drinking may be attributed to its impact on the central nervous system. *GRIK4* is also involved in the glutamatergic synapse and neuro-active ligand-receptor interaction pathways. Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system, and has important physiologic significance for neural plasticity and opioid dependence. It is interesting that the neuroactive ligand-receptor interaction pathway contains both *OPRM1* and *GRIK4* found in the present study. *OPRM1*, a typical candidate gene for alcohol consumption, has been reported to be associated with alcoholism in Asian, American, and European populations [29, 30]. Apart from morphine addiction and neuroactive ligand-receptor interaction pathways, *OPRM1* also plays a role in the estrogen signaling pathway. Alcohol is known to increase aromatization, so moderate alcoholic beverage consumption and serum estradiol levels are closely related. Moderate alcohol consumption is an important factor for postmenopausal estrogen status [31], and it is well documented that estrogen levels closely relate to BMD in females [32]. The effects of estrogens on trabecular versus cortical bone mass are mediated by direct effects on osteoclasts and osteoblasts. The protection of cortical bone mass by estrogens is mediated via estrogen receptor α [33]. One important mechanism by which estrogen inhibits bone resorption is that estrogen can inhibit the production of specific cytokines (e.g., IL-1, IL-6), thus inhibiting osteoclast formation. IL-1 receptor antagonist is a protein that binds to IL-1 receptors and inhibits the binding of IL-1 α and IL-1 β [34]. Several published studies that focused on bone development related pathways or gene networks implicated *IL1RN* as having an effect on BMD or osteoporosis. Mutations in *IL1RN* have been identified in monogenic autoinflammatory bone disorders that have allowed more detailed dissection of immunologic defects that can produce sterile osteomyelitis [35]. Allelic variation at the *IL1RN* locus is associated with differential rates of early postmenopausal bone loss in the spine [36]. At the same time, there is growing evidence for the involvement of

proinflammatory cytokines in alcohol dependence. The *IL1RN*1* allele was overrepresented in Spaniards with alcoholism, and also the *IL1RN*1/_511* haplotype was more frequent in the Spaniards with alcoholism than in the controls [37].

Several potential mechanisms may help to explain how *IL1RN* and *OPRM1* can serve as pleiotropic candidate genes affecting both BMD and alcohol drinking in females. The nervous system and the immune system are two-way loop adjusting processes, which may explain potential pleiotropic effects of genes that are involved in either of the two systems. Cytokines are pleiotropic proteins that coordinate the host inflammatory response, and also mediate signaling between cells of nonimmune tissues, including the nervous system [38]. It is well known that several members of the cytokine family are present in the brain. IL-1 activity in the brain has been related to the differentiation of dopaminergic neurons and the modulation of central monoaminergic activity [39]. A variety of mechanisms have been proposed to explain how opioid use and abuse suppress the immune system [40]. Several studies have proved that opioids can reduce the proliferation of bone marrow cells [41].

One limitation of the study is that we cannot quantitatively determine the phenotype alcohol drinking. Instead, we graded the alcohol drinking as 1, 2, 3, 4, 5, or 6, representing drinking alcohol never, less than once, once or twice, three to six times, seven to ten times, or more than ten times per week respectively. Although such graded methods are widely used in other studies [15, 42, 43], it will be more informative if the ethanol value can be quantitatively determined, such as in the study of Venkat et al. [44]. In that study, alcohol drinking was converted into a quantitative value—expressed as grams of alcohol per week. We could not do that in this study because of the limitation of the study design.

For all findings in the current study, our results provided prospective evidence of the role of *DYNC2H1*, *GRIK4*, *OPRM1*, and *IL1RN* in affecting alcohol drinking or BMD. Specifically, the current study identified these genes as pleiotropic genes underlying variation of both BMD and alcohol drinking by a powerful bivariate GWAS. Identifying the pleiotropic genes that influence the risk of both BMD and alcohol consumption will inform our knowledge of the joint action of multiple mechanisms underlying the disorders, potentially improving their diagnosis, treatment, and prevention. The findings enhance our knowledge of genetic associations between BMD and alcohol consumption, and provide a rationale for subsequent functional studies of these implicated genes in the pathophysiologic mechanisms of diseases/traits, such as alcohol dependence and osteoporosis.

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Fig. 1. The distribution of bivariate P values across genome in the Caucasian sample. P values are plotted according to their physical positions on successive chromosomes. The red line in the distribution of bivariate P values of male spine bone mineral density (BMD) and alcohol drinking corresponds to $P = 5 \times 10^{-8}$ (color figure online)

Fig. 2.

Bivariate associations of four SNP groups with bone mineral density (BMD)–alcohol drinking in *DYNC2H1*, *GRIK4*, *IL1RN*, and *OPRM1*. The linkage disequilibrium between two SNPs is standardized D' ($D/D_{\max}$)

Table 1

Basic characteristics of the study participants

	Male	Female
Number of participants	506	1553
Number of drinkers	377	1171
Alcohol drinking level	2.04 ± 1.77	1.52 ± 1.34
Age (years)	50.13 ± 16.02	51.37 ± 12.76
Hip BMD (g/cm ²)	1.03 ± 0.146	0.94 ± 0.14
Spine BMD (g/cm ²)	1.07 ± 0.15	1.01 ± 0.15
Whole body BMD (g/cm ²)	1.18 ± 0.11	1.09 ± 0.10

Alcohol drinking level, age, hip bone mineral density (BMD), spine BMD, and whole body BMD are presented as the mean ± standard deviation

Table 2

Interesting SNPs of bivariate/univariate association for spine bone mineral density (BMD) and alcohol drinking in males

SNP	Chromosome	Chromosome position	Gene	Predicted function	Allele ^a	MAF	Bivariate <i>P</i>		Univariate <i>P</i>	
							Alcohol drinking–spine BMD	Alcohol drinking–hip BMD	Alcohol drinking–whole body BMD	Spine BMD
rs685395	11	103196731	<i>DYNC2H1</i>	Intronic	T C	0.145	1.944×10^{-8}	4.911×10^{-3}	1.251×10^{-1}	0.775
rs657752	11	103258399	<i>DYNC2H1</i>	Intronic	T C	0.075	8.567×10^{-7}	3.108×10^{-1}	2.639×10^{-1}	0.507
rs614902	11	103230517	<i>DYNC2H1</i>	Intronic	A T	0.075	1.271×10^{-6}	2.391×10^{-1}	2.171×10^{-1}	0.502
rs682851	11	103230083	<i>DYNC2H1</i>	Intronic	A G	0.075	1.402×10^{-6}	2.575×10^{-1}	2.158×10^{-1}	0.614
rs626330	11	103165062	<i>DYNC2H1</i>	Intronic	T G	0.076	1.712×10^{-6}	3.011×10^{-1}	2.666×10^{-1}	0.356
rs689295	11	103213567	<i>DYNC2H1</i>	Intronic	A G	0.077	5.778×10^{-6}	2.563×10^{-1}	2.265×10^{-1}	0.363
rs625103	11	103186682	<i>DYNC2H1</i>	Intronic	A C	0.073	3.276×10^{-5}	3.511×10^{-1}	1.865×10^{-1}	0.349
rs12284644	11	120450016	<i>GRIK4</i>	Noncoding intronic	G A	0.465	5.660×10^{-5}	2.936×10^{-5}	9.065×10^{-5}	0.764
rs7103821	11	120435024	<i>GRIK4</i>	5' upstream	G T	0.460	9.692×10^{-5}	6.637×10^{-5}	1.663×10^{-5}	0.769

MAF:minor allele frequency

^aThe first allele represents the minor allele of each locus

Table 3

Interesting SNPs of bivariate/univariate association for whole body bone mineral density (BMD) and alcohol drinking in females

SNP	Chromosome	Chromosome position	Gene	Predicted function	Allele ^a	MAF	Bivariate <i>P</i>		Univariate <i>P</i>		
							Alcohol drinking–whole body BMD	Alcohol drinking–hip BMD	Alcohol drinking–spine BMD	Whole body BMD	
<i>rs444413</i>	2	113885826	<i>IL1RN</i>	Intronic	T/C	0.280	1.187 × 10 ^{−5}	8.441 × 10 ^{−3}	1.366 × 10 ^{−2}	0.947	0.821
<i>rs432014</i>	2	113888579	<i>IL1RN</i>	Intronic	G/A	0.280	1.337 × 10 ^{−5}	7.815 × 10 ^{−3}	1.250 × 10 ^{−2}	0.342	0.812
<i>rs423904</i>	2	113887262	<i>IL1RN</i>	Intronic	T/C	0.280	1.453 × 10 ^{−5}	6.600 × 10 ^{−3}	6.623 × 10 ^{−5}	0.987	0.809
<i>rs419598</i>	2	113887207	<i>IL1RN</i>	Coding/intronic	G/A	0.280	1.471 × 10 ^{−5}	4.025 × 10 ^{−3}	7.510 × 10 ^{−3}	0.936	0.863
<i>rs442710</i>	2	113887399	<i>IL1RN</i>	Intronic	A/G	0.280	1.477 × 10 ^{−5}	5.825 × 10 ^{−3}	1.014 × 10 ^{−2}	0.987	0.813
<i>rs447713</i>	2	113887672	<i>IL1RN</i>	Intronic	G/A	0.280	1.477 × 10 ^{−5}	5.825 × 10 ^{−3}	1.014 × 10 ^{−2}	0.987	0.813
<i>rs451578</i>	2	113888557	<i>IL1RN</i>	Intronic	T/C	0.297	4.304 × 10 ^{−5}	1.253 × 10 ^{−2}	2.239 × 10 ^{−2}	0.784	0.852
<i>rs431726</i>	2	113889006	<i>IL1RN</i>	Intronic	G/T	0.273	5.806 × 10 ^{−5}	4.516 × 10 ^{−3}	6.878 × 10 ^{−3}	0.874	0.960
<i>rs4251984</i>	2	113877226	<i>IL1RN</i>	Intronic	G/A	0.271	6.146 × 10 ^{−5}	5.531 × 10 ^{−3}	1.482 × 10 ^{−2}	0.810	0.998
<i>rs9479769</i>	6	154483146	<i>OPRM1</i>	Intronic	C/A	0.447	4.556 × 10 ^{−5}	5.449 × 10 ^{−5}	5.089 × 10 ^{−5}	9.180 × 10 ^{−4}	0.198
<i>rs4870268</i>	6	154472327	<i>OPRM1</i>	Intronic	C/T	0.457	7.512 × 10 ^{−5}	6.630 × 10 ^{−5}	6.433 × 10 ^{−5}	3.850 × 10 ^{−3}	0.447
<i>rs1998221</i>	6	154506647	<i>OPRM1</i>	Intronic	T/G	0.455	8.677 × 10 ^{−5}	1.033 × 10 ^{−5}	1.014 × 10 ^{−5}	7.980 × 10 ^{−4}	0.311

MAF:minor allele frequency

^aThe first allele represents the minor allele of each locus