Causal Association of Alcohol Consumption with Bone Mineral Density in Korean

: A Mendelian Randomization Analysis

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Abstract

Causal Association of Alcohol Consumption with Bone Mineral Density : A Mendelian Randomization Analysis

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(Directed by Professor Sun Ha Jee)

Background: Bone Mineral Density (BMD) is a diagnostic tool for osteoporosis, which is a systemic bone disease. It is characterized by a decrease in the strength of bone and an increased risk of fracture. With osteoporotic fracture, the quality of life is deteriorated as it causes limitations on routine daily-life, which leads to major problems in health and sometimes results in death. Alcohol consumption is used for prediction of osteoporotic fracture but the impact of alcohol consumption on BMD were reported inconsistently from the observational studies, suggesting that alcohol has negative impact on bone formation directly or indirectly, light drinking is positive on increased bone mass, or alcohol is the secondary factor which may affect on lifestyle so it is not the primary

factor of reduced bone mass. Since the impact of alcohol on bone is controversial, our study was designed to reveal the causal association with Mendelian randomization method using genetic variants and eliminating the methodological limitations of observational studies such as residual, uncontrolled confounding, and reverse causality.

Materials and Methods: Among the data of Korean Genome and Epidemiology Study (KoGES) cohort conducted in Ansan and Ansung province with over 40 years old from 2001 to 2002, this study included total 8,392 participants who had provided the information of genetic variants, alcohol consumption, and the result of BMD with informed consent. Drinking status and drinking amount were used as exposure variable of alcohol consumption. Polymorphism of rs671 in ALDH2 gene was determined as instrument variable. For outcome variable, T-scores of BMD assessed in Distal radius and Mid-Shaft Tibia with Speed of Sound (SOS) method were used. The causal associations between alcohol consumption and BMDs were tested with two-stage least squares method for Mendelian randomization (MR) analysis.

Results: Among 8,392 participants, 4,025 (47.96%) were men and 4,367(52.04%) were women. Current drinker was much higher in men as 71.63% (N=2,883) than in women as 25.28% (N=1,104) and the mean (SD) of drinking amount from current drinkers was 26.86 (31.07) g/day in men and 5.43 (10.86) g/day in women. The effect size (β) of rs671 on drinking amount was confirmed as 7.59 (p <0.001) with the study population and the F-statistics was 281.46. In men, the effect size (β) was 14.54 (p <0.001) with F-statistics= 308.41. For drinking status as exposure, it showed that the number of G allele

in rs671 significantly increased the risk of alcohol consumption as OR=2.91 (p <0.001) in all study population and as OR=4.67 (p <0.001) in men and OR=3.03 (p <0.001) in women. According to the result of MR analysis, the current drinker increased DR-BMD by β =0.370 SD (p=0.002) compared to non-current drinker without adjustment, increased by β =0.344 (p=0.003) adjusted for sex and age, and increased by β =0.292 (p=0.012) adjusted for sex, age, diabetes mellitus, medical history, smoking status, and physical activity. In contrast, there was no causal association between drinking status and MST-BMD. The sub-group analysis with men showed similar results as above, while it didn't suggest any causality with women group in both DR-BMD and MST-BMD. The causal association of drinking amount with BMDs were also analyzed and the result had similar outcomes with drinking status.

Conclusion: Considering the causal association between drinking status/drinking amount and DR-BMD, it implies that current drinker has increased DR-BMD so alcohol consumption seems to be beneficial to bone health. Whereas, MST-BMD showed no association and it was also not detected with women's group at all in both BMDs. Thus, the impact of alcohol on bone health should be interpreted with caution considering the skeletal site and the hormone impact such as estrogen.

Keywords: alcohol consumption, drinking, bone mineral density, Mendelian randomization

I. Introduction

1. Study Background

Bone Mineral Density (BMD) is a diagnostic and prognostic tool to evaluate bone health by measuring bone mass per skeletal unit area (Kains, 2007). Bone is a living tissue that is remodeled during lifetime by the interaction of osteoblasts and osteocytes. During the remodeling process, bone formation is reduced and bone resorption is increased, resulting in a decrease of bone mass, cortical thickness, and the amount and size of the trabecular bone. As a result, it makes bone weak and fragile, leading to bone fracture easily even with minor impacts (Mo et al.,2008). Osteoporosis is a systemic bone disease, characterized by a decrease in the strength of bone and an increased risk of fracture due to reduced bone mass and microstructural changes of bone (Lee and Lee, 2011).

According to the definition of WHO, osteoporosis is diagnosed with T-score of BMD decreased less than -2.5 standard deviations from the normal, and Osteopenia is defined as less than -1 standard deviations in the group above -2.5, and normal as above -1 standard deviation (Kains,2007).

BMD increases rapidly during puberty, reaches to the peak at 25 years of age, maintain it at 35 – 40 years of age, and then decline continuously for 0.3 - 0.5

per year (Lim, Sun and Kim, 20119). So, as a consequence of ageing in modern society, the prevalence of osteoporosis and osteoporotic fracture also grow up.

With osteoporotic fracture, the quality of life is deteriorated as it causes limitations on routine daily-life, which leads to major problems in health and sometimes results in death. Hence, medical and economical burden of osteoporotic fracture or death is increasing with ageing accordingly.

However, diagnosis and treatment of osteoporosis are delayed because osteoporosis itself has no special symptoms (Lee, 2017). Thus, the importance of prevention is becoming more prominent.

Candidate risk factors which might be associated with osteoporosis or fracture have been reported such as age, sex, family history, glucocorticoid use, secondary osteoporosis, prior fragility fracture, low BMI, physical activity, exercise, calcium intake, caffeine, drinking, smoking, postmenopausal status, and fertility (Kains, 2007). Of these risk factors, genetic factors cannot be corrected and the factors such as sex, age, and hormone are also difficult to change. But, manageable factors related to lifestyle such as alcohol consumption, smoking, exercise, diet can be changed to maintain bone density and strength to reduce the risk of osteoporosis and fracture.

In particular, alcohol consumption is used for prediction of osteoporotic fracture but a high intake of alcohol was not included in an assessment guideline for osteoporosis for use in case-finding strategies (Kains, 2007). The impact of

alcohol consumption on BMD were reported inconsistently from the observational studies (Lee and Lee, 2011; Lee and Lee, 1998; Kim et al., 2009; Byeon,2006; Kim and Kweon, 2011; Hyeon et al., 2011; Kim, Lee and Yeo, 2015; Kim and Lee, 2018) and some showed J-curve or U-curve relationship between alcohol consumption and BMD (Fini et al., 2012; Watts et al., 2012; Berg et al., 2008; Kanis et al., 2005).

It is known that alcohol has impact on bone indirectly reducing bone formation by inducing metabolic abnormalities of parathyroid hormone or vitamin D that regulate calcium metabolism, and directly by inhibiting differentiation and growth of osteoblast and promoting bone cell proliferation (Callaci et al., 2004). Studies of alcoholic bone loss have reported that excessive alcohol consumption interferes with bone reconstruction and inhibits new bone formation (Callaci et al., 2004). However, mild drinking has been shown to increase bone mineral density (Cho et al., 2018; Lee et al., 2018). The precise threshold of the beneficial effect was not defined (Berg et al., 2008).

Since the impact of alcohol on bone is controversial, high level of evidence is required to answer the question whether alcohol consumption can be used as a risk factor. However, all of the observational studies suffer from several methodological limitations, which are prone to residual or uncontrolled confounding, measurement errors, and reverse causality. Hence, it is difficult to demonstrate causality using information from observational studies alone.

Recently, there was new analysis method known as Mendelian Randomization (MR) which investigates causality using genetic information as an instrument variable (IV). Once genetic variants are chosen as proxies for the exposure of interest, the association between exposure and outcome can be evaluated indirectly (Smith and Ebrahim, 2003; Haycock et al., 2016; Davey and Hemani, 2014). Genetic variants are not likely to be associated with environmental factors because it is assorted randomly at meiosis. Hence, it is expected that MR design mitigates the effect of confounding, bias and eliminates reverse causations, which are generally mentioned as limitations of conventional observational studies (Smith and Ebrahim, 2003).

With theses advantages of MR design, there were several MR studies which performed to identify potential causal association of BMD (Larsson, Michaëlsson and Burgess, 2018). Since rs671 which is a SNP associated with alcohol consumption has been identified (Jorgenson et al., 2017), there has also been a research evaluating association between alcohol consumption and BMD with European population using that genetic variant (Guo, Wu and Fu, 2018). The result of the study suggested that there was no association between alcohol and BMD. However, the causality in Asian, especially for Korean, was not revealed. Thus, the aim of this study was to investigate the causal association between alcohol consumption and BMD in Korean by using genetic variant as instrumental variable in a Mendelian randomization analysis.

2. Study Objective

The primary objective of this study is to investigate whether alcohol consumption is casually associated with Bone Mineral Density (BMD) with Mendelian Randomization method using Korean Genome and Epidemiology Study (KoGES) data.

The detailed objectives of this study are as follows;

1) To estimate the epidemiological association between alcohol consumption and bone mineral density using KoGES data
2) To test the hypothesis that alcohol consumption is not causally associated with BMD, which can be inferred from the results of Speed of Sound test – it could be assessed by adopting genetic variants

II. Materials and Methods

1. Study design

The study design of the study is as below. (Figure 1)

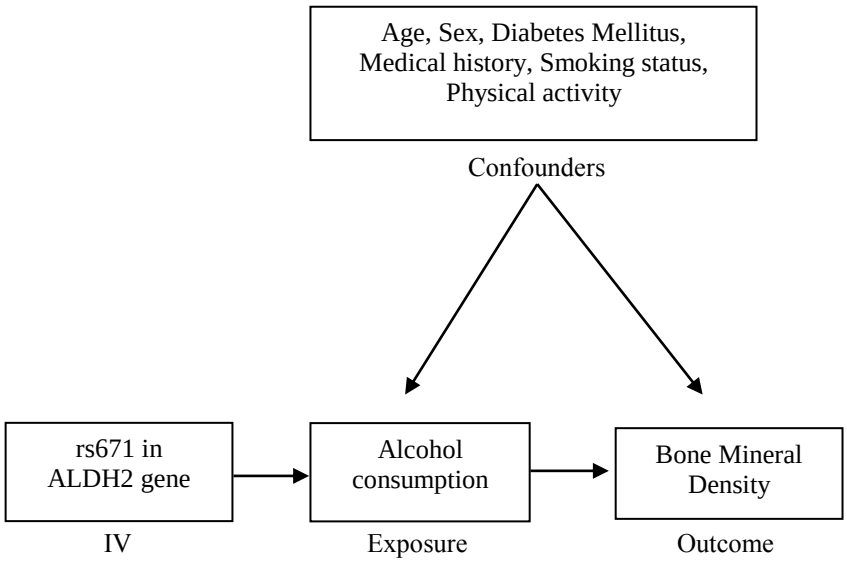

Figure 1. Study design

2. Study Population

Korean genome and epidemiology study (KoGES) is a large prospective cohort initiated by government, which aims to identify the genetic-environmental factors of chronic diseases in Korean and their interaction with the general population over the age of 40. The study consists of three population-based cohorts and three gene-environment model cohorts constructed to identify risk factors for genetic-environmental interactions of chronic diseases. Of theses six types of studies, the data from a population-based cohorts conducted in Ansan and Ansung province was used for this study. Data collection was executed in 2001-2002 through surveys and examinations for epidemiological data such as health, lifestyle, the result of blood and urine test, genetic information etc. More detailed descriptions about the design of KoGES cohort and characteristics have been previously published (Kim and Han, 2017). Among the participants from 2001 to 2002, the number of subjects who had provided informed consent for the study was 10,030. Within this population, participants who satisfied all of following conditions: people who had essential variables including genetic information, alcohol consumption, and the result of BMD test were included in the study. Finally, total 8,392 participants were selected with 4,025 men and 4,367 women (Figure 2).

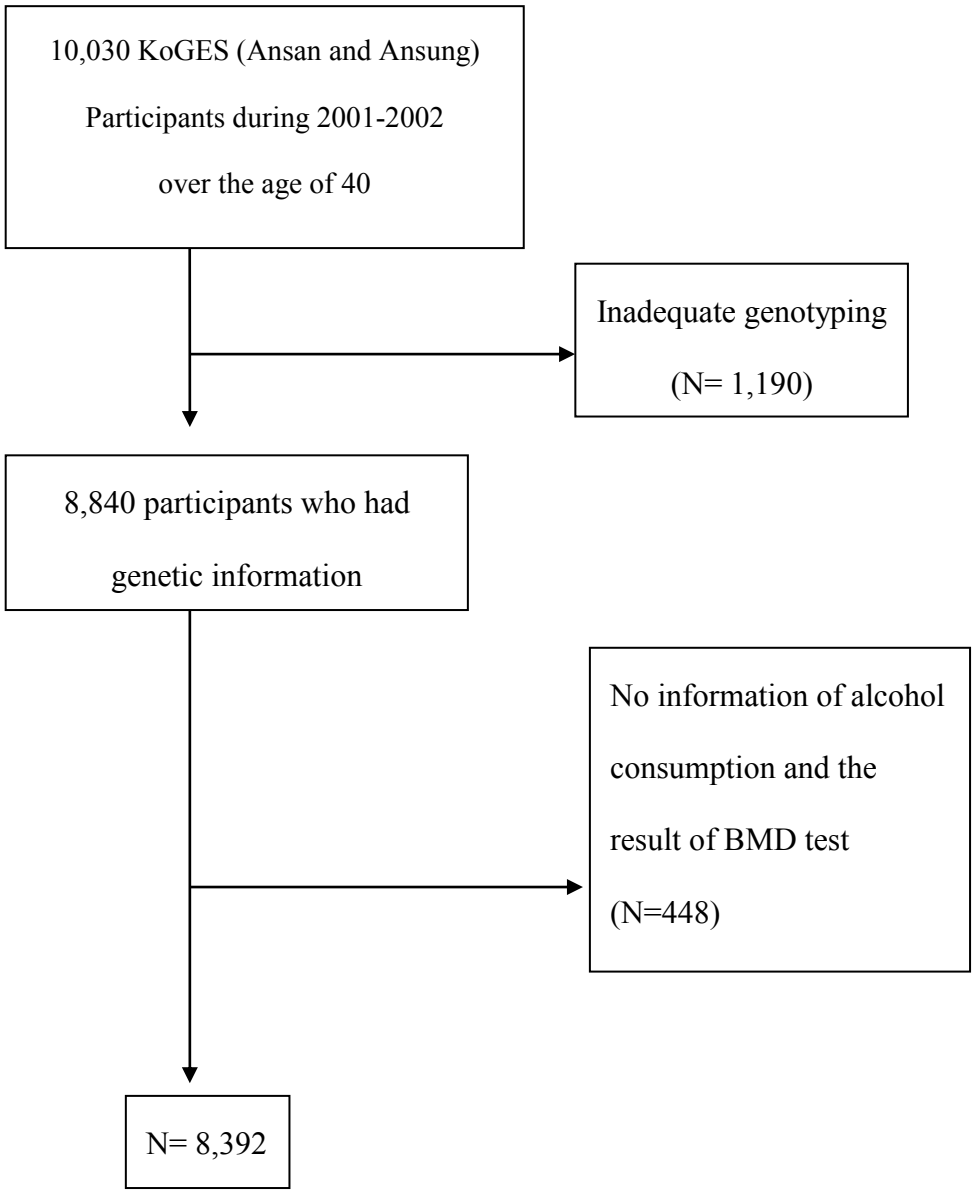

Figure 2. Flow Chart of study population

3. Data collection

The participants voluntarily responded to an interview, a questionnaire administrated by trained staff and physical examination at the hospitals and health institutions. Through survey, the data of demographics, medical history, concomitant medication, family history, smoking behavior, alcohol consumption, physical activities, social activities, psychosocial factors, and dietary habit were collected. Physical examination was also taken for blood pressure, pulse, weight, height, waist circumference (WC), electrocardiogram, chest X-ray, bone mineral density (BMD) test, and clinical laboratory test etc.

Exposure - Alcohol consumption

The questionnaire included the questions on alcohol consumption to check whether a participant is Never drinker, Former drinker, and Current drinker. Total year of drinking was also provided and the drinking amount was collected with various type of drinks. So, the variable of the daily amount of drinking (g/day) was created based on the formulation below;

※ The sum of the amount of drinking form Rice wine, Beer, Refined rice wine, Wine, Korean distilled spirits, liquor

- Proportion of ethanol =0.7893

- alcohol content per each type of drink (%): Rice wine (6), Beer (4.5), Refined rice wine (15), Wine (13), Korean distilled spirits (22), Formal spirits (40)

In this study, drinking status and drinking amount (g/day) were used as exposure variable for alcohol consumption. Drinking status was categorized into current drinker or non-current drinker which includes never drinker and former drinker.

Covariates

In several studies (Lee, 2017; Byeon, 2006; Chang et al., 2006; National Institutes of Health, 2001; Jeon and Kim, 2016, Napoli N et al., 2017), risk factors associated with lower BMD were suggested; older age, sex (women), estrogen deficiency, low weight and body mass index, diabetes mellitus, previous history of bone fracture, the parental history of osteoporosis, current smoking, glucocorticoids use, co-morbidity of rheumatoid arthritis and secondary osteoporosis, and drinking over 3 units (approx. 8-10 g of alcohol/unit) per day. In contrast, physical activities such as exercise were shown as positive association with increased BMD.

Among theses factors, the variables of age, sex, medical history of rheumatoid arthritis, Diabetes Mellitus, BMI (calculated as weight divided by the square of height), WC (the average of WC examined for 3 times), Smoking status, Physical activities (more than medium level in hardness in their daily life such as brisk

walking, swimming, mowing, hiking, sports game, running etc.) were taken as covariates in this study, which were available from KoGES data.

4. Definition of Outcome

The outcome of interest was the bone mineral density (BMD), which is a diagnosis tool for osteoporosis. The result of BMD is expressed with two types of diagnostic criteria; T-score and Z-score. Firstly, T-score has been used by the World Health Organization are based on the standard deviation calculated by comparing the bone marrow of the hip, lumbar or forearm of the patient with the normal mean value of the young adult control group. Secondly, Z-score is used to compare the bone density with the patients of similar conditions. T-score and Z-score are not similar when the population of the reference database is different.

Regarding to skeletal sites, BMD of Hip and Spine are generally measured to predict osteoporosis and/or fracture. But, the results of BMD in Distal Radius and Mid-Shaft Tibia sites were only available from KoGES data, which were assessed with Speed of Sound (SOS) method.

Thus, T-score of Distal radius and Mid-Shaft Tibia were used as outcome variable in our study.

5. DNA Extraction and IV selection

From 10,030 KoGES (Ansan and Ansung) participants during 2001-2002, SNPs information were obtained from 8,840 samples with peripheral blood, which were genotyped with Affymetrix Genome-Wide Human SNP Array 5.0. 1,190 samples were removed due to low call rates, contamination, sex inconsistencies, cryptic relatedness, or serious concomitant illness (Cho et al., 2009).

IV selections and generic risk scores

We searched genetic variants manually through literatures and found out some study results revealed genetic variants related to alcohol consumption. Among them, SNP (rs671 in ALDH2 gene) was used as only one instrument variable in the study since it has shown the strongest association with both of drinker status (OR=0.40, $p=2.28\times10^{-72}$) and the drinking amount per week (β = - 0.17, $p=5.42\times10^{-4}$) in East Asians (Jorgenson et al., 2017).

6. Statistical analysis

The participants who provided exposure and outcome variables with genetic information were included in the analysis. Drinking status (current drinker, non-current drinker) was used as exposure variables for alcohol consumption.

For outcome variable, T-scores of BMD measured in Distal radius (DR-BMD) and Mid-Shaft Tibia (MST-BMD) were analyzed respectively.

All of the analysis were carried out with subgroup by sex in addition to overall participants.

A 2-sided significance level of α =0.05 was used. All statistical analyses were performed using SAS 9.4 and STATA/IC 13.1 (Stata Corp LP, College Station, TX).

A. Descriptive analysis

The general characteristics of the participants were presented with descriptive statistics for overall participants and subgroups by sex. Categorical variables such as diabetes mellitus, drinking status, smoking status, medical history, physical activities were shown with frequencies and proportions. Means and standard deviation (SD) were provided for continuous variables such as age, BMI, WC, drinking amount, and BMD.

In order to estimate the effect of genetic variants on exposures, ordinary least square (OLS) regression and logistic regression were performed respectively.

The associations of genetic variants with exposure, outcome, and confounding variables were statistically tested with ANOVA test for continuous variables and Chi-square test for categorical variables for the three genotypes of rs671.

B. Conventional regression

Observational multivariable regression was performed to elucidate the association of alcohol consumption with BMD, while adjusting for confounding variables.

In order to fit the best model, adjustments on confounders were done with three types of model based on the combination of confounders;

- Model 1: none (crude)
- Model 2: Sex, Age
- Model 3: Sex, Age, Diabetes Mellitus, Medical history, Smoking status, Physical activity,

C. Mendelian randomization analysis

Mendelian randomization analysis was performed in three steps (Figure 3) with Two-Stage Least Squares method (2SLS). Firstly, logistic regression for drinking status and linear regression for drinking amount were used to assess the strength of the association between genetic variant and exposure to determine whether the association was fully mediated by exposure. The strength of the association between genetic variant and exposure was expressed as F statistics. We used the regression analysis under the assumption of an additive genetic model. Secondly, we examined the association of genetic variants with participants' characteristics to confirm whether there is no association between confounders and instrumental variables. Thirdly, the association between outcome (BMD) and exposure

(alcohol consumption), which were expressed with genetic variants, was tested by multivariable linear regression model.

D. Sensitivity analyses

Durbin-Wu-Hausman test was carried out for assess Endogeneity.

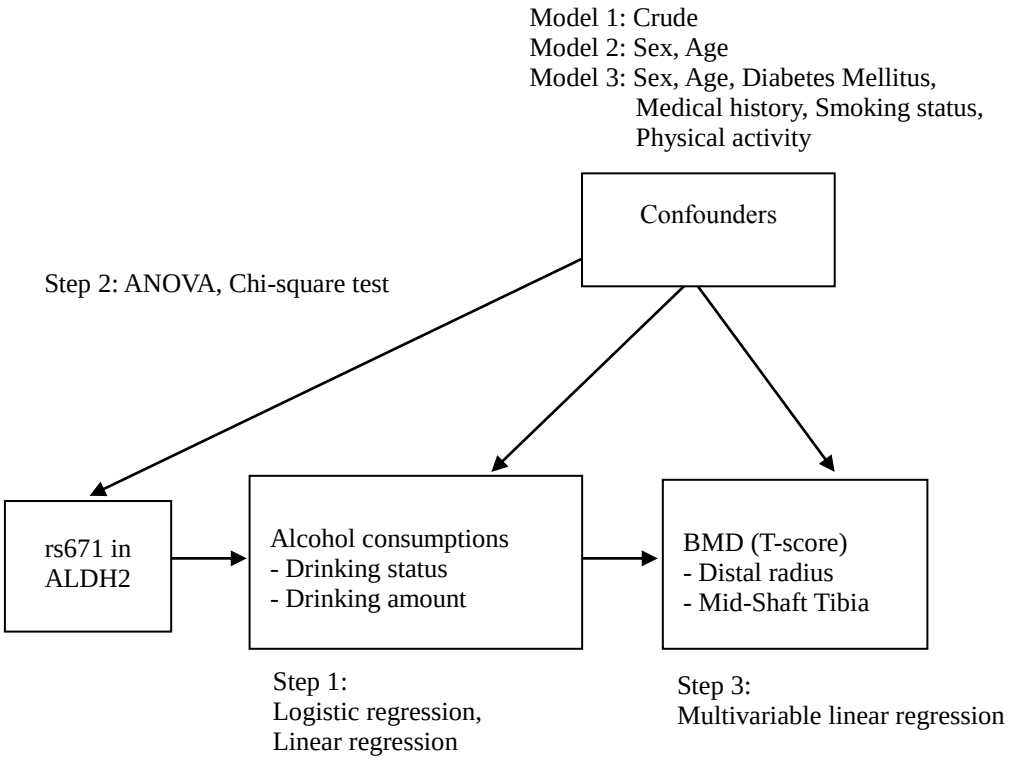

Figure 3. Flowcharts of analyses

7. Ethics statement

This study was approved by an Institutional Review Board of Graduate school of Public Health, Yonsei University [IRB no: 4-2015-1132].

III. Results

1. General characteristics of study population

The total participants (N=8,392) was included in the descriptive analysis. The majority of sex was women (52.04%, n=4,367) but it is almost similar with men (47.96%, n=4,025). The mean (SD) of Age, BMI, WC, Smoking, drinking amount, and BMDs were showed as 52.31 (8.93) year-old, 24.57 (3.10) kg/m^2, 82.71 (8.77) cm, 21.02 (28.73) g/day, 0.13 (1.42) SD in DR-BMD, -0.37 (1.50) SD in MST-BMD, respectively.

The portion of current drinkers in each respective sex was much higher in men (71.63%, n=2,883) than women (25.28 %, n=1,104). In the same context with drinking, the percentage of current smokers was 49.48% (n=1,987) in men and 3.6% (n=155) in women. Among those current drinkers, it demonstrated that men drink alcohol much more than women as the mean (SD) of drinking amount was 26.86 (31.07) g/day in men and 5.43 (10.68) g/day in women.

However, women showed lower BMD in both skeletal sites as 0.10 (0.02) SD in DR-BMD and -0.94 (1.56) SD in MST-BMD, while these were 0.16 (0.02) and 0.24 (1.16) respectively in men.

The portion of medical history of rheumatoid arthritis was low as 5.05% (n=419) in all participants and it was mostly women (n=344). For Diabetes Mellitus, only 587 participants (7%) had this comorbidity.

59.68 % (n=4,912) of study population didn't do physical activities more than medium level in hardness in their daily life but 28.08 % (n=2,311) had physical activities more than or equal to 90 minutes. The rest of them had physical activities for less than 30 min. in 5.05% (n=416), 30 to 59 min. in 4.31 % (n=355), and 60 to 89 min. in 2.88 % (n=237).

All of the characteristics were statistically significant in difference between men and women.

The details were presented in [Table 1].

Table 1. General Characteristics

Characteristics	Total (N=8392)		Men (N=4025)		Women (N=4367)		p-value ‖
	Mean (SD)						
Age (year)	52.31	(8.93)	51.78	(8.80)	52.79	(9.01)	<.0001
BMI (kg/m^2)	24.57	(3.10)	24.23	(2.93)	24.89	(3.22)	<.0001
WC(cm)*	82.71	(8.77)	83.65	(7.65)	81.84	(9.61)	<.0001
Drinking amount (g/day)	21.02	(28.73)	26.86	(31.07)	5.43	(10.86)	<.0001
DR-BMD (SD)†	0.13	(1.42)	0.16	(0.02)	0.10	(0.02)	0.0272
MST-BMD (SD)†	-0.37	(1.50)	0.24	(1.16)	-0.94	(1.56)	<.0001
	N (%)						
Diabetes Mellitus	8,388		4,023		4,365		0.018
No	7,801	(93.00)	3,705	(92.10)	4,096	(93.84)	
Yes	587	(7.00)	318	(7.90)	269	(6.16)	
Drinking Status	8,392		4,025		4,367		<.0001
Non-current drinker	4,405	(52.49)	1,142	(28.37)	3,263	(74.72)	
Current drinker	3,987	(47.51)	2,883	(71.63)	1,104	(25.28)	
Smoking status	8,325		4,016		4,309		<.0001
Never	4,858	(58.35)	762	(18.97)	4,096	(95.06)	
Former	1,325	(15.92)	1,267	(31.55)	58	(1.35)	
Current	2,142	(25.73)	1,987	(49.48)	155	(3.60)	
Medial History‡	8,304		3,971		4,333		<.0001
No	7,885	(94.95)	3,896	(98.11)	3,989	(92.06)	
Yes	419	(5.05)	75	(1.89)	344	(7.94)	
Physical activity§	8,231		3,953		4,278		<.0001
No	4,912	(59.68)	2,253	(56.99)	2,659	(62.16)	
< 30 min	416	(5.05)	231	(5.84)	185	(4.32)	
30 - 59 min	355	(4.31)	186	(4.71)	169	(3.95)	
60 – 89 min	237	(2.88)	128	(3.24)	109	(2.55)	
>90 min	2,311	(28.08)	1,155	(29.22)	1,156	(27.02)	

*: Waist circumstance
†: T-score of BMD, SD: standard deviation, DR: distal radius, MST: mid-shaft tibia
‡: Medical history of rheumatoid arthritis
§: More than medium level in hardness such as brisk walking, swimming, mowing, hiking, sports game, running etc.
‖ : ANOVA test for continuous variables and Chi-square test for categorical variables.

2. SNPs associated with exposure variables

After literature search, rs671 SNP in ALDH2 gene was determined which was revealed from previously reported genome-association studies to be associated with alcohol consumption in East Asian.

The effect size of rs671 on drinking amount was confirmed as β =7.59 (p <0.001) and the F-statistics was 281.46 in the whole study population, indicating the drinking amount was increased by 7.59 g/day when the effect allele added by one. In subgroup, it was presented as β = 14.54 (p <0.001) with F-statistics= 308.41 in men and β = 1.09 (p <0.001) with F-statistics= 42.9 in women.

For drinking status as exposure variable, it showed that the genetic variant increased the probability of being current drinker as OR=2.91 (p <0.001) in all study population, OR=4.67 (p <0.001) in men, and OR=3.03 (p <0.001) in women.

It implies that rs671 can explain the exposure variable strong enough.

The details were presented in [Table 2] for all study population and [

Table **3**] in men.

Table 2. Genetic variants used as instruments of alcohol consumption in all study population

SNP	Chr	Gene	Minor allele	MAF	Drinking status				Drinking amount (g/day)			
					OR	95% CI		p-value	F	β	SE	p-value
rs671	12	ALDH2	A	0.17	2.91	2.65	3.18	<.0001	281.46	7.59	0.45	<.0001

SNP: Single Nucleotide Polymorphism, Chr: Chromosome, MAF: Minor Allele Frequency, OR: Odds Ratio, CI: Confidence Interval, SE: Standard Error

Table 3. Genetic variants used as instruments of alcohol consumption in men

SNP	Chr	Gene	Minor allele	MAF	Drinking status				Drinking amount (g/day)			
					OR	95% CI		p-value	F	β	SE	p-value
rs671	12	ALDH2	A	0.17	4.67	4.08	5.35	<.0001	308.41	14.54	0.83	<.0001

SNP: Single Nucleotide Polymorphism, Chr: Chromosome, MAF: Minor Allele Frequency, OR: Odds Ratio, CI: Confidence Interval, SE: Standard Error

Table 4. Genetic variants used as instruments of alcohol consumption in women

SNP	Chr	Gene	Minor allele	MAF	Drinking status				Drinking amount (g/day)			
					OR	95% CI		p-value	F	β	SE	p-value
rs671	12	ALDH2	A	0.17	3.03	2.56	3.59	<.0001	42.9	1.09	0.17	<.0001

SNP: Single Nucleotide Polymorphism, Chr: Chromosome, MAF: Minor Allele Frequency, OR: Odds Ratio, CI: Confidence Interval, SE: Standard Error

3. Associations of intermediate phenotype, outcome and potential confounders with genetic variables

The association of rs671 with exposure, outcome or confounders were tested and it resulted that DR-BMD and WC were statistically significant. In men's group, physical activity and diabetes mellitus was also shown significance in addition to DR-BMD and WC.

It potentially suggested that there are associations between rs671 and the other confounders as well as alcohol consumption.

The details were presented in [Table 5] for all study population, [Table 6] in men, and [Table 7] in women.

Table 5. Associations of intermediate phenotype, outcome and potential confounders with genetic variables in all study population

Characteristics	Polymorphism rs671 for alcohol consumption						
	AA	(n=237)	GA	(n=2403)	GG	(n=5752)	p-value*
	Mean (SD)						
Age (year)	52.63	(8.83)	52.38	(9.)	52.26	(8.9)	0.7257
BMI (kg/m^2)	24.42	(3.23)	24.48	(3.06)	24.62	(3.12)	0.1399
WC (cm) *	81.50	(8.6)	82.20	(8.84)	82.97	(8.74)	<.0001
Drinking amount (g/day)	6.60	(9.02)	13.52	(23.98)	22.88	(29.52)	<.0001
DR-BMD (SD) †	-0.02	(1.49)	0.07	(1.38)	0.16	(1.43)	0.0092
MST-BMD (SD) †	-0.47	(1.56)	-0.38	(1.51)	-0.36	(1.49)	0.5237
	N (%)						
Sex	237		2,403		5,752		0.4603
Men	122	(51.48)	1,138	(47.36)	2,765	(48.07)	
Women	115	(48.52)	1,265	(52.64)	2,987	(51.93)	
Diabetes Mellitus	237		2,401		5,750		0.2229
No	223	(94.09)	2,249	(93.67)	5,329	(96.68)	
Yes	14	(5.91)	152	(6.33)	421	(7.32)	
Drinking Status	237	(100.)	2,403	(100.)	5,752	(100.)	<.0001
Non-current drinker	216	(91.14)	1,650	(68.66)	2,539	(44.14)	
Current drinker	21	(8.86)	753	(31.34)	3,213	(55.86)	
Smoking status	233		2,383		5,709		
Never	133	(57.08)	1,397	(58.62)	3,328	(58.29)	0.9741
Former	36	(15.45)	380	(15.95)	909	(15.92)	
Current	64	(27.47)	606	(25.43)	1,472	(25.78)	
Medial History ‡	235		2,377		5,692		0.4724
No	226	(96.17)	2,264	(95.25)	5,395	(94.78)	
Yes	9	(3.83)	113	(4.75)	297	(5.22)	
Physical activity §	232		2,358		5,641		0.186
No	136	(58.62)	1,440	(61.07)	3,336	(59.14)	
< 30 min	11	(4.74)	117	(4.96)	288	(5.11)	
30 - 59 min	8	(3.45)	80	(3.39)	267	(4.73)	
60 – 89 min	7	(3.02)	77	(3.27)	153	(2.71)	
>90 min	70	(30.17)	644	(27.31)	1,597	(28.31)	

*: Waist circumstance

†: T-score of BMD, SD: standard deviation, DR: distal radius, MST: mid-shaft tibia

‡: Medical history of rheumatoid arthritis

§: More than medium level in hardness such as brisk walking, swimming, mowing, hiking, sports game, running etc.

ǁ : ANOVA test for continuous variables and Chi-square test for categorical variables.

Table 6. Associations of intermediate phenotype, outcome and potential confounders with genetic variables in men

Characteristics	Polymorphism rs671 for alcohol consumption						
	AA	(n=122)	GA	(n=1138)	GG	(n=2765)	p-value ‖
	Mean (SD)						
Age (year)	51.97	(8.91)	51.93	(8.84)	51.71	(8.78)	0.7657
BMI (kg/m^2)	23.87	(3.01)	24.16	(2.96)	24.27	(2.92)	0.2273
WC (cm) *	81.86	(7.54)	83.22	(7.92)	83.90	(7.53)	0.0013
Drinking amount (g/day)	7.55	(9.57)	16.36	(26.20)	29.69	(31.70)	<.0001
DR-BMD (SD) †	0.02	(1.14)	0.08	(1.16)	0.21	(1.24)	0.0039
MST-BMD (SD) †	0.20	(1.25)	0.24	(1.16)	0.24	(1.16)	0.9405
	N (%)						
Diabetes Mellitus	122		1,137		2,764		0.0261
No	118	(96.72)	1,060	(93.23)	2,527	(91.43)	
Yes	4	(3.28)	77	(6.77)	237	(8.57)	
Drinking Status	122		1,138		2,765		<.0001
Non-current drinker	104	(85.25)	555	(48.77)	483	(17.47)	
Current drinker	18	(14.75)	583	(51.23)	2,282	(82.53)	
Smoking status	121		1,135		2,760		0.8591
Never	25	(20.66)	204	(17.97)	533	(19.31)	
Former	36	(29.75)	366	(32.25)	865	(31.34)	
Current	60	(49.59)	565	(49.78)	1,362	(49.35)	
Medial History ‡	121		1,122		2,728		0.1113
No	117	(96.69)	1,108	(98.75)	2,671	(97.91)	
Yes	4	(3.31)	14	(1.25)	57	(2.09)	
Physical activity §	119		1,118		2,716		<.0001
No	64	(53.78)	659	(58.94)	1,530	(56.33)	
< 30 min	4	(3.36)	74	(6.62)	153	(5.63)	
30 - 59 min	4	(3.36)	34	(3.04)	148	(5.45)	
60 – 89 min	4	(3.36)	37	(3.31)	87	(3.20)	
>90 min	43	(36.13)	314	(28.09)	798	(29.38)	

*: Waist circumstance
†: T-score of BMD, SD: standard deviation, DR: distal radius, MST: mid-shaft tibia
‡: Medical history of rheumatoid arthritis
§: More than medium level in hardness such as brisk walking, swimming, mowing, hiking, sports game, running etc.
‖ : ANOVA test for continuous variables and Chi-square test for categorical variables.

Table 7. Associations of intermediate phenotype, outcome and potential confounders with genetic variables in women

Characteristics	Polymorphism rs671 for alcohol consumption						
	AA	(n=115)	GA	(n=1265)	GG	(n=2987)	p-value ‖
	Mean (SD)						
Age (year)	53.34	(8.72)	52.79	(9.13)	52.77	(8.98)	0.8014
BMI (kg/m^2)	25.00	(3.37)	24.76	(3.11)	24.93	(3.26)	0.2561
WC (cm) *	81.13	(9.61)	81.27	(9.50)	82.10	(9.65)	0.0266
Drinking amount (g/day)	0.04	(0.27)	0.50	(3.32)	1.71	(6.67)	<.0001
DR-BMD (SD) †	-0.07	(1.79)	0.07	(1.56)	0.12	(1.59)	0.3445
MST-BMD (SD) †	-1.18	(1.54)	-0.94	(1.57)	-0.92	(1.55)	0.2116
	N (%)						
Diabetes Mellitus	115		1,264		2,986		0.4989
No	105	(91.30)	1,189	(94.07)	2,802	(93.84)	
Yes	10	(8.70)	75	(5.93)	184	(6.16)	
Drinking Status	115		1,265		2,987		<.0001
Non-current drinker	112	(97.39)	1,095	(86.56)	2,056	(68.83)	
Current drinker	3	(2.61)	170	(13.44)	931	(31.17)	
Smoking status	112		1,248		2,949		0.6778¶
Never	108	(96.43)	1,193	(95.59)	2,795	(94.78)	
Former	0	(0.00)	14	(1.12)	44	(1.49)	
Current	4	(3.57)	41	(3.29)	110	(3.73)	
Medial History ‡	114		1,255		2,964		0.3544
No	109	(95.61)	1,156	(92.11)	2,724	(91.90)	
Yes	5	(4.39)	99	(7.89)	240	(8.10)	
Physical activity §	113		1,240		2,925		0.4352
No	72	(63.72)	781	(62.98)	1,806	(61.74)	
< 30 min	7	(6.19)	43	(3.47)	135	(4.62)	
30 - 59 min	4	(3.54)	46	(3.71)	119	(4.07)	
60 – 89 min	3	(2.65)	40	(3.23)	66	(2.26)	
>90 min	27	(23.89)	330	(26.61)	799	(27.32)	

*: Waist circumstance
†: T-score of BMD, SD: standard deviation, DR: distal radius, MST: mid-shaft tibia
‡: Medical history of rheumatoid arthritis
§: More than medium level in hardness such as brisk walking, swimming, mowing, hiking, sports game, running etc.
‖ : ANOVA test for continuous variables and Chi-square test for categorical variables.
¶: Fisher's exact test

4. Results of conventional regression and Mendelian randomization analyses

The statistics data for the associations of alcohol consumption with two types of BMD included to obtain estimates of the effects of alcohol consumption on BMD through Mendelian randomization as well as conventional regression (OLS).

A. Association between drinking status and two types of BMD

In all study population, drinking status showed significantly positive association with DR-BMD in all models through OLS as well as MR. That is, current drinker increased DR-BMD by 0.370 SD compared to non-current drinker in crude model 1. It was raised by 0.344 SD in model 2 adjusted for sex and age, by 0.292 SD in model 3 adjusted for diabetes mellitus, medical history, smoking status, physical activity in addition to model 2.

For MST-BMD, Model 1 had significance of positive association with drinking status in OLS only, while MR didn't show any association.

[Table 8],[Table 9]

In men's group, the result also suggested that current drinker increased DR-BMD by 0.365 SD in model 1, by 0.362 SD in model 2, and by 0.315 SD in model 3. All models showed significance in both OLS and MR. However, MST-BMD couldn't show positive association significantly in model 1 via OLS but other

models in OLS as well as MR results didn't have significant association with drinking status. [Table 10],[Table 11]

In women's group, only model 1 showed positive association in OLS but MR results had no significant causal associations in both of DR-BMD and MST-BMD for all models.
[Table 12],[Table 13]

Table 8. Association of drinking status with DR-BMD using Conventional Regression and Mendelian Randomization analysis in all study population (n=8,303)

	Observational Multivariable regression			Mendelian Randomization Analysis			
	β	SE	*p*	β	SE	*p*	Endogeneity test (P)
Model 1	0.217	0.031	< 0.001	0.370	0.121	0.002	0.188
Model 2	0.071	0.034	0.037	0.344	0.116	0.003	0.013
Model 3	0.077	0.034	0.025	0.292	0.116	0.012	0.052

Model 1: crude
Model 2: adjusted for Sex, Age
Model 3: model 2 plus additional adjustments for Diabetes Mellitus, Medical history, Smoking status, Physical activity

Table 9. Association of drinking status with MST-BMD using Conventional Regression and Mendelian Randomization analysis in all study population (n=8,345)

	Observational Multivariable regression			Mendelian Randomization Analysis			
	β	SE	*p*	β	SE	*p*	Endogeneity test (P)
Model 1	0.694	0.032	< 0.001	0.120	0.127	0.344	< 0.001
Model 2	0.031	0.033	0.352	0.096	0.113	0.394	0.544
Model 3	0.030	0.034	0.377	0.096	0.114	0.398	0.541

Model 1: crude
Model 2: adjusted for Sex, Age
Model 3: model 2 plus additional adjustments for Diabetes Mellitus, Medical history, Smoking status, Physical activity

Table 10. Association of drinking status with DR-BMD using Conventional Regression and Mendelian Randomization analysis in men (n=4,007)

	Observational Multivariable regression			Mendelian Randomization Analysis			
	β	SE	*p*	β	SE	*p*	Endogeneity test (P)
Model 1	0.088	0.042	0.039	0.365	0.112	0.001	0.007
Model 2	0.073	0.043	0.089	0.362	0.112	0.001	0.005
Model 3	0.074	0.043	0.085	0.315	0.110	0.004	0.017

Model 1: crude
Model 2: adjusted for Age
Model 3: model 2 plus additional adjustments for Diabetes Mellitus, Medical history, Smoking status, Physical activity

Table 11. Association of drinking status with MST-BMD using Conventional Regression and Mendelian Randomization analysis in men (n=4,017)

	Observational Multivariable regression			Mendelian Randomization Analysis			
	β	SE	*p*	β	SE	*p*	Endogeneity test (P)
Model 1	0.082	0.041	0.043	0.017	0.106	0.871	0.509
Model 2	0.062	0.041	0.127	0.012	0.106	0.911	0.609
Model 3	0.046	0.042	0.270	0.020	0.106	0.850	0.791

Model 1: crude
Model 2: adjusted for Age
Model 3: model 2 plus additional adjustments for Diabetes Mellitus, Medical history, Smoking status, Physical activity

Table 12. Association of drinking status with DR-BMD using Conventional Regression and Mendelian Randomization analysis in women (n=4,296)

	Observational Multivariable regression			Mendelian Randomization Analysis			
	β	SE	*p*	β	SE	*p*	Endogeneity test (P)
Model 1	0.382	0.055	0.000	0.379	0.274	0.166	0.989
Model 2	-0.012	0.050	0.811	0.333	0.242	0.169	0.144
Model 3	-0.025	0.051	0.620	0.273	0.252	0.279	0.225

Model 1: crude
Model 2: adjusted for Age
Model 3: model 2 plus additional adjustments for Diabetes Mellitus, Medical history, Smoking status, Physical activity

Table 13. Association of drinking status with MST-BMD using Conventional Regression and Mendelian Randomization analysis in women (n=4,328)

	Observational Multivariable regression			Mendelian Randomization Analysis			
	β	SE	*p*	β	SE	*p*	Endogeneity test (P)
Model 1	0.296	0.054	0.000	0.311	0.271	0.251	0.954
Model 2	-0.072	0.049	0.143	0.280	0.242	0.248	0.136
Model 3	-0.075	0.051	0.139	0.301	0.253	0.234	0.127

Model 1: crude
Model 2: adjusted for Age
Model 3: model 2 plus additional adjustments for Diabetes Mellitus, Medical History, Smoking status, Physical activity

B. Association between drinking amount and two types of BMD

In all study population, drinking amount showed significantly positive association with DR-BMD in all models through MR as well as OLS. That is, DR-BMD was increased by 0.012 SD when the drinking amount is increased by 1 g/day in crude model 1. It was raised by 0.011 SD in model 2 adjusted for sex and age, by 0.009 SD in model 3 adjusted for diabetes mellitus, medical history, smoking status, physical activity in addition to model 2.

For MST-BMD, Model 1 had significance of positive association with drinking amount in OLS only, while MR didn't show any association.

[Table 14], [Table 15]

In men's group, the result also suggested that drinking amount (g/day) increased DR-BMD by 0.008 SD in model 1, by 0.008 SD in model 2, and by 0.007 SD in model 3. All models with DR-BMD showed significance in MR but not OLS. Since the endogeneity tests were all significant, the result of MR could be accepted rather than OLS.

For MST-BMD, association was not shown at all in both of OLS and MR.

[Table 16],[Table 17]

In women's group, only model 1 showed positive association in OLS but MR results had no significant causal associations in both of DR-BMD and MST-BMD.

[Table 18],[Table 19]

Table 14. Association of drinking amount with DR-BMD using Conventional Regression and Mendelian Randomization analysis in all study population (n=8,127)

	Observational Multivariable regression			**Mendelian Randomization Analysis**			
	β	**SE**	***p***	**β**	**SE**	***p***	**Endogeneity test (P)**
Model 1	0.002	0.001	0.030	0.012	0.004	0.003	0.008
Model 2	-0.001	0.001	0.419	0.011	0.004	0.004	0.002
Model 3	-0.001	0.001	0.277	0.009	0.004	0.018	0.008

Model 1: crude
Model 2: adjusted for Sex, Age
Model 3: model 2 plus additional adjustments for Diabetes Mellitus, Medical history, Smoking status, Physical activity

Table 15. Association of drinking amount with MST-BMD using Conventional Regression and Mendelian Randomization analysis in all study population (n=8,168)

	Observational Multivariable regression			**Mendelian Randomization Analysis**			
	β	**SE**	***p***	**β**	**SE**	***p***	**Endogeneity test (P)**
Model 1	0.012	0.001	0.000	0.003	0.004	0.419	0.035
Model 2	0.000	0.001	0.767	0.002	0.004	0.520	0.473
Model 3	0.000	0.001	0.698	0.002	0.004	0.541	0.481

Model 1: crude
Model 2: adjusted for Sex, Age
Model 3: model 2 plus additional adjustments for Diabetes Mellitus, Medical history, Smoking status, Physical activity

Table 16. Association of drinking amount with DR-BMD using Conventional Regression and Mendelian Randomization analysis in men (n=3,896)

	Observational Multivariable regression			**Mendelian Randomization Analysis**			
	β	**SE**	***p***	**β**	**SE**	***p***	**Endogeneity test (P)**
Model 1	0.001	0.001	0.414	0.008	0.003	0.001	0.001
Model 2	0.000	0.001	0.599	0.008	0.003	0.001	0.001
Model 3	0.000	0.001	0.901	0.007	0.002	0.005	0.003

Model 1: crude
Model 2: adjusted for Age
Model 3: model 2 plus additional adjustments for Diabetes Mellitus, Medical history, Smoking status, Physical activity

Table 17. Association of drinking amount with MST-BMD using Conventional Regression and Mendelian Randomization analysis in men (n=3,904)

	Observational Multivariable regression			**Mendelian Randomization Analysis**			
	β	**SE**	***p***	**β**	**SE**	***p***	**Endogeneity test (P)**
Model 1	0.001	0.001	0.120	0.000	0.002	0.909	0.579
Model 2	0.001	0.001	0.251	0.000	0.002	0.847	0.602
Model 3	0.000	0.001	0.674	0.000	0.002	0.865	0.766

Model 1: crude
Model 2: adjusted for Age
Model 3: model 2 plus additional adjustments for Diabetes Mellitus, Medical history, Smoking status, Physical activity

Table 18. Association of drinking amount with DR-BMD using Conventional Regression and Mendelian Randomization analysis in women (n=4,231)

	Observational Multivariable regression			**Mendelian Randomization Analysis**			
	β	**SE**	***p***	**β**	**SE**	***p***	**Endogeneity test (P)**
Model 1	0.013	0.004	0.002	0.051	0.042	0.223	0.357
Model 2	-0.002	0.004	0.570	0.048	0.037	0.195	0.166
Model 3	-0.003	0.004	0.472	0.039	0.038	0.298	0.258

Model 1: crude
Model 2: adjusted for Age
Model 3: model 2 plus additional adjustments for Diabetes Mellitus, Medical history, Smoking status, Physical activity

Table 19. Association of drinking amount with MST-BMD using Conventional Regression and Mendelian Randomization analysis in women (n=4,264)

	Observational Multivariable regression			**Mendelian Randomization Analysis**			
	β	**SE**	***p***	**β**	**SE**	***p***	**Endogeneity test (P)**
Model 1	0.011	0.004	0.009	0.047	0.041	0.260	0.378
Model 2	-0.004	0.004	0.300	0.045	0.037	0.220	0.173
Model 3	-0.003	0.004	0.423	0.047	0.038	0.212	0.174

Model 1: crude
Model 2: adjusted for Age
Model 3: model 2 plus additional adjustments for Diabetes Mellitus, Medical history, Smoking status, Physical activity

C. Summary of result

Overall, alcohol consumption including drinking status (current drinker, non-current drinker) and drinking amount (g/day) showed positive causal associations with DR-BMD in all study population and the subgroup of men, whereas women didn't suggest any causal relationship. There was no association observed between alcohol consumption with MST-BMD. [Table 20]

The effect size varies on adjustments in both of methods; Conventional regression and Mendelian randomization as described in [Table 21][Table 22].

Table 20. Causal association between alcohol consumption and BMDs

Alcohol consumption	DR-BMD			MST-BMD		
	ALL	Men	Women	ALL	Men	Women
Drinking status (current drinker)	+	+	-	-	-	-
Drinking amount (g/day)	+	+	-	-	-	-

+: positive association, -: no association

Table 21. Effect size in conventional regression

OLS			DR-BMD			MST-BMD		
			ALL	Men	Women	ALL	Men	Women
Alcohol consumption	Drinking Status (current drinker)	Model 1	0.217*	0.088*	0.382*	0.694*	0.082*	0.296*
		Model 2	0.071*	0.043	-0.012	0.031	0.062	-0.072
		Model 3	0.077*	0.043	-0.025	0.030	0.046	-0.075
	Drinking Amount (g/day)	Model 1	0.002*	0.001	0.013	0.012*	0.001	0.011*
		Model 2	-0.001	0.000	-0.002	0.000	0.001	-0.004
		Model 3	-0.001	0.000	-0.003	0.000	0.000	-0.003

*: p <0.05

Table 22. Effect size in Mendelian randomization

MR			DR-BMD			MST-BMD		
			ALL	Men	Women	ALL	Men	Women
Alcohol consumption	Drinking Status (current drinker)	Model 1	0.370*	0.365*	0.379	0.120	0.017	0.311
		Model 2	0.344*	0.362*	0.333	0.096	0.012	0.280
		Model 3	0.292*	0.315*	0.273	0.096	0.020	0.301
	Drinking Amount (g/day)	Model 1	0.012*	0.008*	0.051	0.003	0.000	0.047
		Model 2	0.011*	0.008*	0.048	0.002	0.000	0.045
		Model 3	0.009*	0.007*	0.039	0.002	0.000	0.047

*: p <0.05, underline: endogeneity test p <0.05

IV. Discussion

1. Discussion of Study Results

A. Review of previous studies and biological plausibility: BMD

Bone is constantly remodeled itself and is hardened by mineralization with calcium. The process is executed with osteocytes, osteoclasts and osteoblasts and their activation is managed by cytokine, hormone, or etc. Through bone remodeling, bone is repaired from damage or stress. The rate of bone turnover is high in childhood because bone formation exceeds resorption and then it becomes balanced in young adulthood. After that time, bone lost started with aging. The decrease of mechanical properties of bone such as collagen, matrix, density, etc. results in bone disease like osteoporosis or osteopenia (Datta et al., 2008). Osteoporosis is diagnosed with bone mineral density (BMD) lower than -2.5 standard deviations. According to WHO report, osteoporotic fracture is the main problem of low BMD which is mostly occurs at the spine, wrist, and hip so BMD is generally measured for Hip and Spine to predict osteoporosis and/or fracture (Kains, 2007).

In our study, the data of BMD which was available from KoGES was the result of measurement with SOS machine, which is not common method to diagnose osteoporosis with the criteria of -2.5 T-score and it can be used only at Distal Radius and Mid-Shaft Tibia not hip or spine. Thus, it has partial limitations to predict accurate association between BMD and risk factors.

B. Review of previous studies and biological plausibility: Alcohol consumption

Alcohol is considered as risk factor for osteoporosis frequently as it impairs bone metabolism by decreasing bone resorption as well as reducing bone formation (Callaci et al., 2004; Turner, 2000; Gong and Wezeman, 2004). In some studies, it was reported that alcohol depresses the ability to differentiate of mesenchymal stem cells in the bone marrow and its migration to osteoblasts resulting in alcohol-induced osteonecrosis (Suh et al., 2005) and it decreases the level of serum osteocalcin that plays a role of bone formation (Nielsen et al., 1990). Furthermore, serum sclerostin was raised in alcoholic patients, which is an endogenous inhibitor of osteoblast function, differentiation and survival leading to decrease of bone mass (Gonzalez-Calvín et al., 1993).

In contrast, some studies proposed that moderate alcohol consumption like two or three unit per day may have positive association with BMD by inhibiting bone resorption and stimulating bone formation (Papadakis, Ganotakis, and Mikhailidis, 2000; Wosje and Kalkwarf, 2007; Rapuri et al., 2000; Jugdaohsingh et al., 2006).

Based on biological researches, the precise mechanism of alcohol to BMD are unclear since it has shown both of positive and negative effect on bone. On the other hand, some researches suggested that alcohol consumption is considered as secondary factor by having effect on the change of lifestyle rather than direct

affecting bone density because drinkers tend to be characterized with lack of exercise, malnutrition, and less calcium intake than non-drinkers (Mikosch, 2014; Hefferan et al., 2003; Lunt et al., 2001; Lau et al., 2001; Johnston and McGovern, 2004; Wang et al., 2002; Huuskonen et al., 2001). That is, the association between alcohol and BMD is still controversial.

2. Discussion of Study Methods

Mendelian Randomization (MR) is a new method to identify causal association using genetic variants in epidemiologic studies. It seems to become popular since this method reduce the limitations of observational studies such as confounding and reverse causations. In order to ascertain the causality through MR, it should satisfy the three assumptions; 1) the genetic variants strongly be associated with exposure so that it can be used as instrument variable, 2) the outcome should be explained through exposure of interest without direct association with the genetic variants, 3) genetic variants should be independent from confounders affecting on outcomes, otherwise the result can be biased.

In this study, although rs671 showed enough power to enable accurate estimation, we couldn't neglect the possibility of pleiotropy bias as it is the one of the limitations of MR design making the estimated causality invalid.

Nevertheless, the study is meaningful since the risk factors widely mentioned in guidelines but inconsistently resulted in many epidemiological studies were tested with genetic variants.

3. Comprehensive Discussion

Alcohol consumption is considered as risk factor to bone fracture as its biological impact on bone metabolism has been researched suggesting that alcohol impairs bone metabolism by decreasing bone resorption as well as reducing bone formation.

However, the association of alcohol consumption with BMD were inconsistently reported from several observational studies. Some studies resulted that light drinker showed increased BMD (Nam et al., 2006), whereas light drinker presented higher risk of osteopenia than heavy drinker with OR=1.41 (95% CI: 1.02-1.93) and higher risk of osteoporosis as OR=3.43 (95% CI: 1.48-7.94) in a study (Kim and Lee, 2018). On the other hand, some articles suggested it has U-shape effect meaning that moderate alcohol consumption may have a positive impact by increasing BMD but excessive consumption is risk factor reducing BMD (Fini et al., 2012; Watts et al., 2012; Berg et al., 2008; Kanis et al., 2005). However, there were also many results which couldn't find any association between alcohol and BMD (Lee and Lee, 2011; Lee and Lee,1998; Kim et al., 2009; Byeon, 2006; Chang et al., 2006; Kim and Kweon, 2011; Hyeon et al., 2016; Kim, Lee and Yeo, 2015).

Moreover, the MR study conducted with European population also couldn't show the causal association between alcohol consumption and BMD (Guo, Wu, and Fu, 2018).

In this study, the possible causal association between alcohol consumptions and DR-BMD was shown regardless of the adjustments of confounders, indicating current drinker increased DR-BMD by 0.370 SD compared to non-current drinker in crude model 1. It was raised by 0.344 SD in model 2 adjusted for sex and age, and by 0.292 SD in model 3 adjusted for diabetes mellitus, medical history, smoking status, physical activity in addition to model 2. In men, it has similar trend as it resulted that it increased by 0.365 SD in model 1, by 0.362 SD in model 2, 0.315 in model 3. They were all significant.

Considering the requirements of MR analysis, the strength of IV is crucial to show significance of estimates and the effect size of SNP to exposure. Since F statistics of rs671 was 281.46 in all study population and 308.41 in men, it implies that the rs671 is strong enough to explain alcohol consumption.

Nevertheless, several limitations were detected in out study. Firstly, it is well known that osteoporosis tends to be inherited from family (Lee and Lee, 2011). So, GWASs have been conducted to reveal polymorphisms affecting osteoporosis. (Gregson et al., 2018; Chesi et al., 2017). And polymorphism of rs671 inducing ALDH2-deficient leads to decrease in bone formation with or without alcohol consumption in animal studies (Shimizu et al., 2011; Himes,

Wezeman and Callaci, 2008; Hoshi et al., 2012). Thus, pleiotropy needs to be confirmed precisely.

Secondly, the exposure of alcohol consumption couldn't reflect the other factors such as the exact amount of drinking and the period of drinking etc. It was mostly analyzed in a type of binary exposure like current drinker or non-current drinker in this study. As U-shape results have been presented from previous studies, further studies are required based on the amount of drinking and/or the period of drinking.

Thirdly, the outcome variable was not collected with the widespread method. Dual energy X-ray absorptiometry (DXA) is the most widely used to measure BMD (Kains, 2007) but the data of BMD of this study was measured with Speed of Sound (SOS) which tests not only bone mineral density but also elasticity, cortical thickness, and micro-architecture. Even though SOS uses WHO criteria and standard T- and Z-scores with high accuracy and precision, it is not recommended to diagnose osteoporosis with T-score and predict osteoporotic fracture because there was no standard information of bone density in Korea population with the method (Kim and Lee, 2013). Thus, we used T-score as it without attempt to diagnose osteoporosis. Furthermore, SOS can be used for peripheral skeletal sites only such as distal radius. Although BMD of Hip and Spine have better predictive ability for fracture, there are several studies

supporting the measurement of bone density at any site had similar predictive ability for decrease in bone mass (Marshall, Johnell and Wedel, 1996).

Fourthly, there is a possibility of misled outcome due to the limitations from study data since the variables were answered with self-reported questionnaire causing recall bias or missing data. For example, the half of the study population was women over 40 year-olds who might went through menopause. Even though menopause is crucial information affecting bone health, it was not collected through self-questionnaire so there might be potential bias. The general characteristic of this study supports the assumption because women showed lower BMDs than men's value, whereas men were more exposed to risk factors such as drink and smoking. Moreover, the majority experienced rheumatoid arthritis was women (82%) which is also well-known symptoms after menopause. So, the subgroup analysis was carried out for men additionally to avoid the bias from missing information. As a consequence, there are limitations on estimating causal association even though we have strong genetic variants.

Thus, further studies are required for better understanding of the causal association between alcohol consumption and BMD with more appropriate data.

V. Conclusion

The total participants (N=8,392) aged over 40 were included in this study from KoGES cohort data. The proportion of sex was 52.04% from women (n=4,367) and 47.96% from men (N=4,025). The mean (SD) of age was 52.31 (8.93) year-old. The percentage of current drinker was much higher in men as 71.63% (N=2,883) than in women as 25.28% (N=1,104) and the mean (SD) of drinking amount from current drinkers was 26.86 (31.07) g/day in men and 5.43 (10.86) g/day. However, the value of BMD in both skeletal sites were also higher in men than women as it presented that T-score of DR-BMD was 0.16 (0.02) SD in men and 0.10 (0.02) in women, and T-score of MST-BMD was 0.24 (1.16) SD in men and -0.94 (1.56) SD in women. We assumed that the lower BMD was shown in women because they were over 40 year-olds and possibly went through menopause. The other factors such as BMI, Waist circumference, Diabetes mellitus, smoking status, and physical activity showed statistical difference in sex but the mean or the proportion was similar between men and women. Some key risk factors affecting bone health such as estrogen deficiency, previous history of bone fracture, the parental history of osteoporosis, and medication history of glucocorticoids couldn’t be included since that information were not collected or rarely reported.

As instrument variable, we determined rs671 in ALDH2 gene through literature search. The effect size of rs671 on drinking amount was confirmed with the study population as β =7.59 ($p < 0.001$) and the F-statistics was 281.46 in the whole study population, indicating the drinking amount was increased by 7.59 g/day when the effect allele added by one. For drinking status as exposure variable, it showed that the genetic variant increased the probability of being current drinker as OR=2.91 ($p < 0.001$) in all study population, OR=4.67 ($p < 0.001$) in men, and OR=3.03 ($p < 0.001$) in women.

The above results imply that rs671 is strong enough to explain the exposure and the minor allele of rs671 has an effect on reducing alcohol consumption.

The association of intermediate phenotype, outcome and potential confounders with genotype of rs671 were tested. It presented that waist circumference and DR-BMD were potentially associated with genotype as well as alcohol consumption. In men, diabetes mellitus and physical activity were also shown to be associated with it. So, the analysis to evaluate the association between alcohol consumption and BMD were carried out with three types of modeling by adjusting the confounders.

According to the result from OLS and MR, positive causal association was suggested between alcohol consumption and DR-BMD regardless of adjustments with confounders.

In MR analysis, the current drinker increased DR-BMD by 0.370 (p=0.002) SD compared to non-current drinker in crude model 1, by 0.344 (p=0.003) SD in model 2 adjusted for sex and age, and by 0.344 (p=0.003) SD in model 3 adjusted for sex, age, diabetes mellitus, medical history, smoking status, and physical activity. In contrast, there was no causal association between drinking status and MST-BMD. Men has the same result with all study population but women didn't have significant association in both of DR-BMD and MST-BMD with drinking status.

The association of drinking amount with BMDs were also analyzed additionally and the results were similar with drinking status. That is, DR-BMD was increased by 0.012 SD when the drinking amount is increased by 1 g/day in model 1, by 0.011 SD in model 2, and by 0.009 SD in model 3. Whereas, it didn't show any association with MST-BMD. Men has the same result with all study population but women didn't have significant association in both of DR-BMD and MST-BMD with drinking amount.

The outcomes imply that alcohol consumption has increased BMD so it seems to be beneficial to bone health. However, as mentioned above in discussion part, there were several limitations such as pleiotropy and bias driven by data collection. Thus, the cautious interpretation is needed.

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Korean Abstract

멘델리언 무작위배정 분석을 이용한 골밀도와 알코올섭취의 인과성 연구

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배경 및 연구 목적

골밀도는 전신적인 골 질환인 골다공증의 진단 도구이다. 골다공증은 뼈의 강도가 감소하여 골절의 위험이 증가한 상태를 의미한다. 골다공증성 골절이 발생하면 일상 생활이 제한되어 삶의 질이 저하되고 건강에 큰 문제를 야기하거나 때로는 사망을 초래한다. 알코올 섭취는 골다공증성 골절의 예측 인자로 사용되지만 알코올 섭취가 골밀도에 미치는 영향에 대한 연구 결과는 다양하게 보고되었다. 알코올이 뼈 형성에 직접 또는 간접적으로 부정적인 영향을 미치는 것으로 보고되거나, 가벼운 음주가 뼈 질량 증가에 긍정적인 영향을 미치는 것으로 보고되기도 하였다. 반면에 알코올이 뼈에 직접적인 영향을 미치는 것이 아니라 생활 양식에 영향을 주게 됨으로써 골밀도에 영향을 주는 2 차 요인으로 보고되기도 한다. 이와 같이, 알코올 섭취와 골밀도의 연관성에 대하여 논란의 여지가 있기 때문에 본 연구는 유전 변이를 이용한 멘델리언 무작위배정 연구 방법을 통해 인과성을 확인하고자 한다.

연구대상과 방법

2001 년부터 2002 년까지 40 세 이상 대상으로 안산 및 안성에서 실시 된 KoGES 코호트 자료에서 자발적 동의 하에 음주, 골밀도, 유전 정보를 포함한 연구자료를 제공한 8,392 명이 본 연구에 포함되었다. 노출 변수인 알코올 섭취에는 음주 여부와 음주량 변수가 사용되었다. ALDH2 유전자에 있는 rs671가 도구 변수로 사용되었다. 결과 변수로는 SOS (Speed of Sound)로 측정한 요골 원위부(DR-BMD) 및 경골(MST-BMD)의 골밀도 T-점수를 사용하였다. 알코올 섭취와 골밀도의 인과 관계는 2단계 최소 제곱법을 이용한 멘델리언 무작위배정 방법으로 분석하였다.

연구 결과

전체 8,392 명 중 남성은 4,029명 (47.96%), 여성은 4,367명 (52.04%)이었다. 현재 음주자의 비율은 남성이 71.63% (N=2,883)로, 여성 25.28% (N=1,104)보다 현저히 높았다. 현재 음주자의 평균 음주량 남성의 경우 26.86(31.07) g/일, 여성의 경우 5.43(10.86) g/일이었다. 연구 대상자의 음주량에 대한 rs671의 효과 크기는 β = 7.59 (p <0.001), F통계량는 281.46으로 확인되었고, 남성에서 확인했을 때 F통계량=308.41, 효과 크기 β =14.54 (p <0.001), 여성에서는 F통계량=42.9, 효과 크기 β =1.09 (p <0.001)이었다. 음주 여부에 대한 rs671 효과 크기의 경우, 모든 연구 집단에서 OR=2.91 (p <0.001), 남성에서 OR=4.67 (p <0.001), 여성에서 OR =3.03 (<0.001)으로 나타냈다.

멘델리언 무작위배정 분석 결과에 따르면, 현재 음주자가 비현재 음주자 대비 DR-BMD을 0.370 SD (p=0.002) 증가시켰고, 성별과 연령을 보정한 후 0.344 SD (p=0.003) 증가, 성별과 연령 뿐만 아니라 당뇨 여부, 류마티스 관절염 여부, 흡연 여부, 신체활동 상태를 보정한 후에는 0.344 SD (p=0.003) 만큼 증가시켰다. 반면에, 음주 여부와 MST-BMD 간에는 인과관계가 확인되지 않았다. 남성 하위 집단 분석 결과도 위와 비슷하게 나타났으나, 여성에서는 DR-BMD와 MST-BMD

모두에서 유의한 인과관계가 확인되지 않았다.

음주량으로 멘델레언 무작위배정 분석을 진행한 결과, 음주량 1 g/day 증가 시 DR-BMD가 0.012 SD (p=0.003) 증가하였고, 성별과 연령 보정 후 0.011 SD (p=0.004) 증가, 성별과 연령 뿐만 아니라 당뇨 여부, 류마티스 관절염 여부, 흡연 여부, 신체활동 상태를 보정한 후에는 0.009 SD (p=0.018) 만큼 증가시켰다. 남성 하위 집단 분석 결과도 위와 비슷하게 나타났으나, 여성에서는 DR-BMD와 MST-BMD 모두에서 유의한 인과관계가 확인되지 않았다.

결론

알코올 섭취와 BMD의 인과성 분석 결과, 현재 음주자인 경우 및 음주량이 증가할수록 DR-BMD가 증가하는 것으로 나타나 알코올 섭취가 골 건강에 긍정적인 영향을 미치는 것으로 확인되었다. 그러나 MST-BMD와는 아무런 연관성이 확인되지 않았다. 골밀도 측정 부위에 따른 차이와 측정 도구의 민감도에 따라 결과가 달라질 수 있으므로, 본 연구의 결과는 신중히 해석되어야 한다. 또한, ALDH2에 관하여 동물을 대상으로 진행한 연구에 따르면, 알코올 섭취 여부와 관계 없이 rs671에 의한 ALDH2 결핍이 골 형성을 감소시키는 것으로 보고됨에 따라 생물학적 메커니즘도 고려되어야 할 것이다.

핵심어: 알코올 섭취, 음주, 골밀도, 멘델리언 무작위배정