Causal Inference Between Adiponectin, Body Mass Index, and Aminotransferase Levels : a Mendelian Randomization Analysis

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ABSTRACT

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Background:

An association between obesity and hepatocyte injury, or liver disease, has been suggested continuously. Adiponectin's potential role in hepatocyte protection against lipid accumulation, fibrosis, or cirrhosis has also been proposed. Although many studies on the relationship between obesity, adiponectin and liver disease have been conducted, a causal relationship has not been established due to the limitations of observational studies. In the present study, we investigated whether obesity and adiponectin levels are causally associated with abnormal aminotransferases levels by applying a Mendelian randomization study design using the population-based Korean Cancer Prevention Study-II (KCPS-II) Biobank Cohort data.

Materials and Methods:

Among the KCPS-II Biobank subcohort, 3,793 healthy Koreans without the hepatitis virus infection and extremely high aminotransferase levels were included as the study population. Genome-wide association study (GWAS) was performed to select highly associated SNPs with adiponectin levels and body mass index (BMI). Both of single representative SNPs and the weighted genetic risk scores for each intermediate phenotype were used as instrumental variables for the Mendelian randomization analysis. Their causal effects on the liver enzyme abnormalities were evaluated with Wald estimator and two-stage least squares method.

Results:

Seven independent SNPs and rs4783244 in *CDH13* gene associated with adiponectin levels as well as 13 SNPs including rs2030323 of the *BDNF* gene for BMI were selected as genetic instrumental variables from GWAS analysis. All single SNPs and weighted genetic risk scores used in the analysis were strongly associated with each intermediate phenotype. The association between adiponectin level and liver enzyme elevation was found to be insignificant according to Mendelian randomization analysis, which implied that no causal relationship between serum adiponectin level and liver injury could be found. However, there was a significant positive association between BMI and liver enzyme levels in both conservative regression and Mendelian randomization analysis, indicating the causal effect of obesity on liver enzyme elevation, which is representative of liver injury.

Conclusion:

The present study suggests a possible pathogenetic mechanism that starts from excessive adiposity and is linked to liver injury, with obesity as a causal factor and decreased adiponectin levels as an intermediate result rather than a cause. This result implies that loss of adiposity is essential and should be the primary target for preventive and therapeutic approaches regarding obesity-related liver damage or metabolic liver diseases.

Keywords: Adiponectin, Body Mass Index, Liver Function Tests, Mendelian Randomization Analysis, Genetic Risk Score

I. Introduction

1. Study Background

The obesity epidemic has been a major health problem, regardless of a nation's economic status or ethnicity. The incidence of obesity-related disorders or complications has also been rising proportionally. In the field of gastroenterology, nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) are of particular concern due to their increased prevalence and potential to progress to liver fibrosis or advanced liver disease.[1,2] It has long been recognized that obesity, type 2 diabetes, and hepatic steatosis are closely related with insulin resistance as a critical factor involved in their common pathophysiology; fatty liver disease is now considered a hepatic component of metabolic syndrome, or hepatic manifestation of insulin resistance.[2-6]

There are several laboratory tests for evaluating liver health or function. They include: liver enzyme assays such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH); tests for liver synthetic function such as serum albumin level and prothrombin time (PT); and serum bilirubin level as a reflection of liver excretory function.[7] Results from sensitive liver enzyme tests that indicate hepatocyte injury are generally used for assessing liver abnormality in clinical or research settings. AST and

ALT, two important serum aminotransferase or transaminase enzymes, are representative liver enzymes that are elevated because they are released in greater amounts into the bloodstream through the damaged liver cell membranes with increased permeability. The majority of AST is found in the liver, but also found in cardiac muscle, skeletal muscle, kidneys, brain, pancreas, lungs, white and red blood cells; in contrast, ALT predominantly exists in the liver (more than 98%). Therefore, the latter is considered as a more specific indicator of liver injury, rarely confused with changes from other organs.[8]

The association between obesity and hepatocyte injury, or liver disease, has been continuously suggested across a wide age spectrum.[9-15] Along with the obesity, adiponectin, a hormone secreted by adipose tissue and known for having anti-lipogenic and anti-inflammatory effects, has been another issue regarding its potential role in hepatocyte protection against lipid accumulation, fibrosis, and cirrhosis in both human and animal livers.[16-19] Because of these associations, adiponectin has been connected to treatment of obesity-related metabolic diseases including liver disorders. It has been proposed as the final or intermediate therapeutic target of such diseases, or as a monitoring target for treating fatty liver disease.[16,17,19-21]

Although this study first assesses serum aminotransferase levels to evaluate potential liver disease or hepatocyte injury, it is important to note that elevated aminotransferase levels, though not serious, may also indicate other conditions. In addition to having been

widely used to check hepatic function in patients with metabolic syndrome,[22-24] they have also been associated with metabolic syndrome risk and death, diabetes, and cardiovascular disease; they have also been used as a measure of overall health and mortality risk, including mortality from liver diseases.[25-29] Hence, the studies involving liver enzymes as dependent variables may have further implications.

As mentioned above, there have been many studies on the relationship between obesity or adiponectin and abnormal liver function or liver diseases; however, all of them had limitations because they were observational studies. They suggested associations, but could not demonstrate causation. However, in recent decades, such obesity-related etiologies have become increasingly important along with the development of medical technology in treatment modalities for hepatitis virus and malignant tumors. Now it is needed to identify causal metabolic factors for liver cell damage or liver diseases, while distinguishing them from intermediate outcomes of abnormal liver function or related alterations.

Mendelian randomization is a statistical analysis that applies the method of instrumental variable analysis to observational epidemiological study, using genetic information as an instrument.[30] It is accepted as a method to account for the limitations of observational studies and enables the establishment of causal relationships. It also allows for an assessment of pathogenetic and therapeutic implications derived from the study results.

2. Study Objectives

The purpose of this study is to investigate whether adiponectin and obesity are causally associated with abnormal aminotransferase levels by applying a Mendelian randomization study design using the population-based Korean Cancer Prevention Study-II (KCPS-II) Biobank Cohort data.

The detailed objectives of this study were as follows:

1) To search for genetic variations regarding serum adiponectin level and body mass index (BMI) among people declared to be negative in both hepatitis B virus antigen and C virus antibody tests from the KCPS-II subcohort by performing a genome-wide association study (GWAS) using a customized chip.
2) To test the hypothesis that adiponectin and obesity are causally associated with hepatocyte injury, which can be inferred from the results of serum aminotransferases assay – it could be assessed by adopting discovered genetic variations or genetic risk scores as unconfounded surrogates for exposure variables, and performing a Mendelian randomization analysis in comparison to the results from conventional regression analysis.

II. Materials and Methods

1. Study Population

The KCPS-II Biobank consisted of participants' health examination data and blood samples obtained from multiple health promotion centers of hospitals located in Seoul and Gyeonggi province. Data collection started in April 2004 and the number of participating hospitals was gradually increased, having attained a constant contribution from 11 hospitals since 2006. More detailed descriptions about the original KCPS-II cohort design and characteristics have been previously published.[31,32]

Among the health examination participants from 2004 to 2013, the number of subjects who had provided informed consent for the study was 159,844. Within this population, there were 111,511 participants who satisfied all of following conditions: people who were 30 years or older at the time of examination, had no history of cancer, stroke, and cardiovascular disease, had no missing or extremely abnormal values for the essential variables including fasting blood glucose, weight, height, BMI, waist circumference, HDL-cholesterol, AST, ALT, GGT, smoking status, and alcohol drinking status, and enrolled in the study before December 31, 2008. Then we randomly selected 5,000 subjects (3,337 men and 1,663 women) among them for the final subcohort group.

There were 3,839 participants who were negative for both hepatitis B virus antigen and hepatitis C virus antibody tests in the subcohort, and 3,817 of them had adequate genetic information that was obtained through various quality control methods for genotyping (described below). Finally, we excluded 24 subjects with extremely high BMI (over 40 kg/m^2) and liver enzyme levels (AST, ALT over 120 IU/L), resulting in 3,793 (2,483 males and 1,310 females) participants for the final study population (Figure 1).

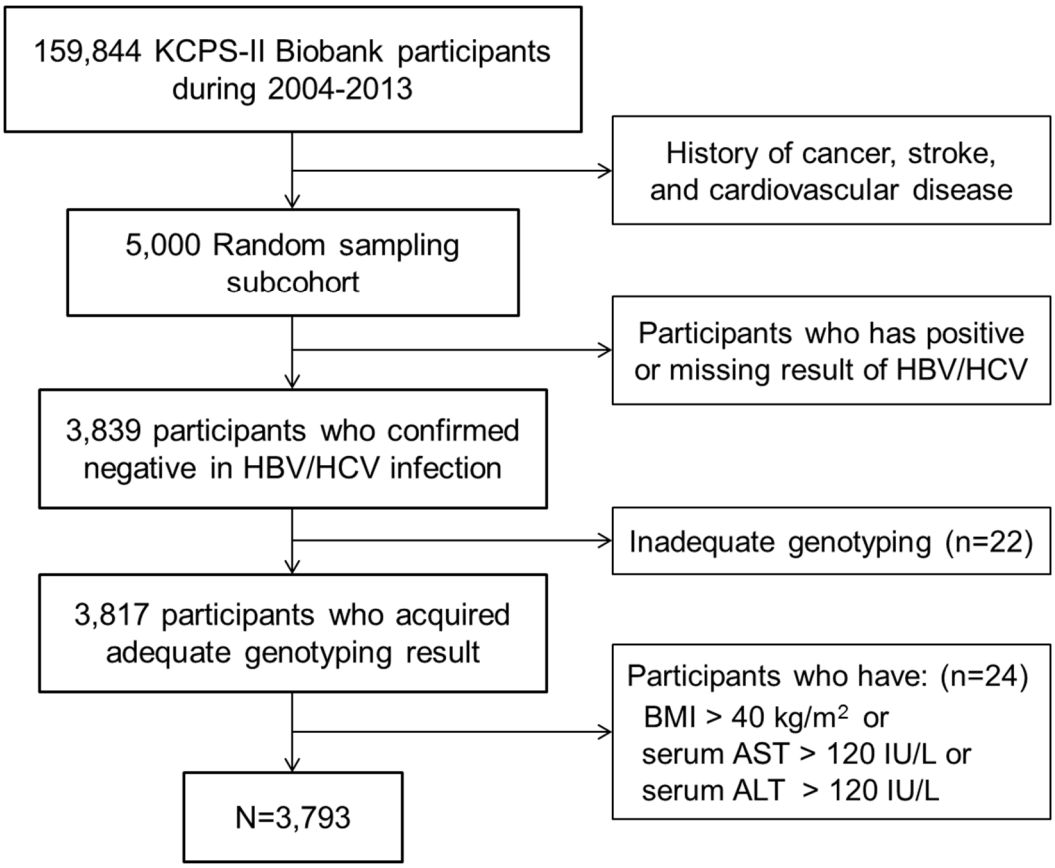

Figure 1. Flow chart describing the study population

2. Data Collection

Each participant was interviewed through a structured questionnaire to collect demographic and behavioral information such as age, sex, cigarette smoking, education, alcohol intake, and exercise status. Participants undertook a medical examination as well; body weight and height were measured while participants were wearing light clothing, BMI was calculated as weight (kg) divided by the square of height (m^2), and waist circumference was measured midway between the lower rib and iliac crest.

For the clinical chemistry assay, serum was separated from peripheral venous blood samples that were obtained from each participant after 12 hours of fasting and was stored at -70°C. Serum biomarkers, including liver enzymes such as AST, ALT, GGT, and LDH were measured using the Histachi-7600 analyzer (Hitachi Ltd., Tokyo, Japan). Adiponectin level was measured using an enzyme-linked immunosorbent assay (Mesdia Co. Ltd., Seoul, Korea). The intra-assay and interassay variances for adiponectin were 6.3% to 7.4% and 4.5% to 8.6%, respectively.[31] Each measurement laboratory had internal and external quality control procedures as required by the Korean Association of Laboratory Quality Control.

3. Genotyping and quality control

Genotyping of DNA was performed with whole blood samples from the KCPS-II Biobank using the Korean Chip (K-CHIP) available through the K-CHIP consortium. A full description about DNA amplification and the genotyping process using the K-CHIP is provided in a previous report.[32] For the last step in the genotyping process, SNPs were identified using the Genotyping Console™ Software (Affymetrix, Santa Clara, CA, USA). Of the 5,000 individuals who underwent genotyping, we first removed samples with a gender mismatch, mutual blood relationship, or had poor genotype data quality such as low genotyping rate. As a result, we obtained a genotype dataset of 3,817 individuals among 3,839 participants who were free of hepatitis B or C viral infection. In performing a GWAS in the final study group (N=3,793), individuals and markers with low call rate (< 0.95), deviation from Hardy-Weinberg equilibrium ($p < 1.0 \times 10^{-4}$), or significantly low minor allele frequency (< 0.01) were also excluded. Therefore 3,780 individuals and 600,712 SNPs were used for a genome-wide association analysis, for the purpose of selecting SNPs highly associated with BMI or adiponectin in our study population.

4. Mendelian randomization analysis

As one of the methodologies that determine causation and resolve noncomparability problems (commonly referred to as confounding) in observational epidemiologic studies, a counterfactual (or potential outcome) model approach has been discussed in great detail for a decade.[33-36] In this model, based on contrary-to-fact conditionals, or hypothetical situations, the causality of risk factors on outcomes can be identified by comparing the exposed individual's outcome to the expected outcome when the same individual is not exposed to a risk; this means all conditions except the exposure to a risk are completely identical in these two settings.

Instrumental variable (IV), a method introduced from econometrics, has been used for making counterfactual causal inference in epidemiological studies.[37,38] The IV, denoted by Z below, was the fourth variable satisfying the following conditions: (1) Z is associated with the exposure of interest X, (2) Z is independent of the confounding factors U (measured and unmeasured) that confound the exposure X – outcome Y relationship, (3) Z is independent of Y given X and all confounders U (measured and unmeasured) of the X – Y association (i.e., 'Z affects outcome Y only through exposure X' or 'Z has no direct effect on Y'). By using such IV that has no relationship with any confounders existing in the study, causal inference between the exposure and outcome became possible. This approach enables control over various confounding factors by

adopting the IV as a surrogate exposure which acts on the outcome only through the exposure variable.

Based on this concept, Mendelian randomization was presented as an approach using genetic markers as the IV.[39-41] It was a method that focused on the law of random assortment (Mendel's second law), which states the inheritance of any trait is independent of the inheritance of other traits, and applied it to the epidemiological setting. In other words, since chromosomes and genes are randomly assigned during reproduction, genetic polymorphism that reflects exposure can be used as the IV independent from possible confounders with the final outcome variable. In this regard, genetic variables to be used must meet the same basic assumptions of the IV as well. Lawlor et al.[30] summarized the three assumptions that the Mendelian randomization approach presupposes as follows:

(1) The genotype is robustly associated with the modifiable (non-genetic) exposure of interest.
(2) The genotype is not associated with confounding factors that bias conventional epidemiological associations between modifiable risk factors and outcomes.
(3) The genotype is related to the outcome only via its association with the modifiable exposure (the assumption also known as 'exclusion restriction').

This Mendelian randomization method that uses a genetic variant for epidemiological causal inference, although there are some limitations that will be discussed later in the

discussion section, also can overcome the reverse causation problem and reduce the inevitable confound of a conventional observational epidemiologic study. In the present work, we estimated the potential causal association between the exposure of interest (X; in this study, serum adiponectin level and BMI) and outcome (Y; in this study, abnormal liver enzyme levels) by using the genetic traits (G; in this study, each single SNP or genetic risk scores) as an instrumental variable (Figure 2).

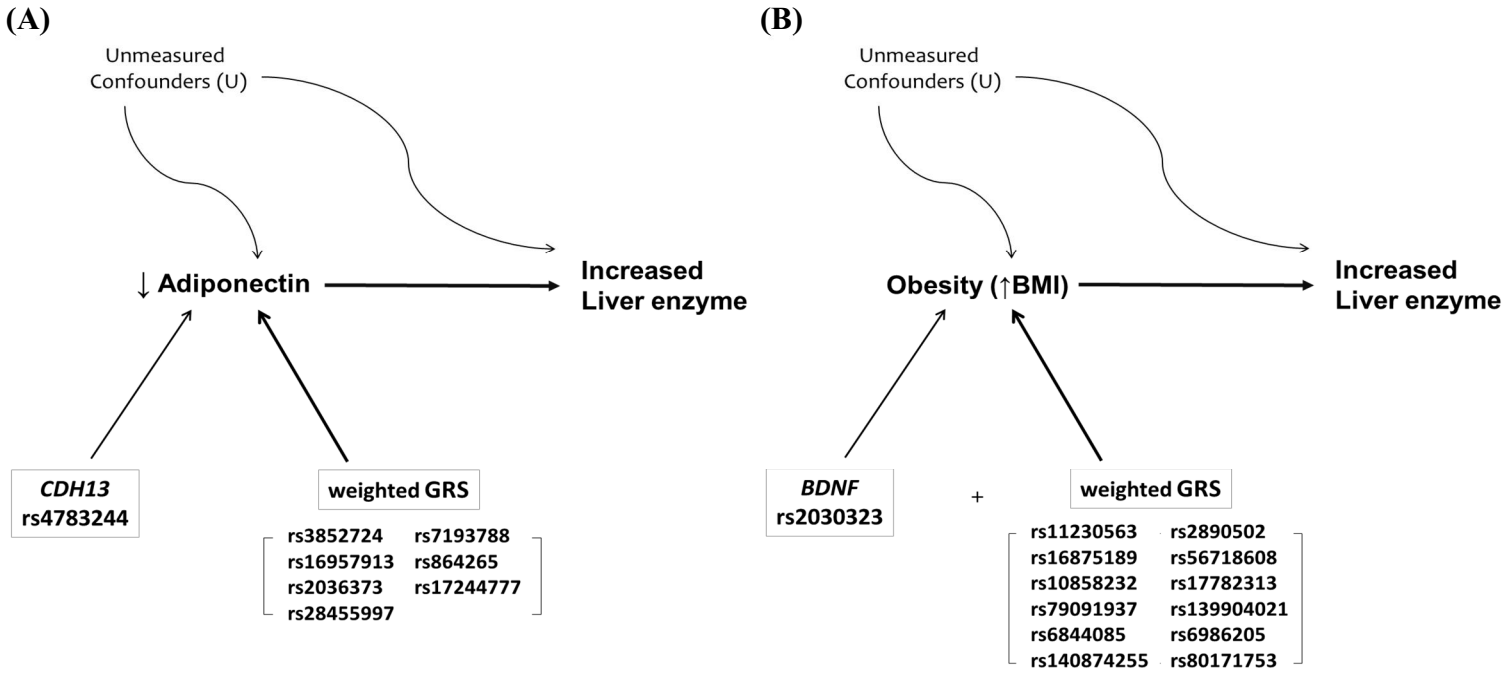

Figure 2. Causal structures encoding the genetic variants as an instrumental variable to estimate the effect of (A) adiponectin and (B) body mass index (BMI) on the elevation in liver enzyme levels

5. IV selection and constructing genetic risk scores

We performed a GWAS with each explanatory variable, serum adiponectin level and BMI. Of the top 20 SNPs that were highly associated with adiponectin levels or BMI, we selected the single representative SNP in each trait (as the first IV) based on *p*-value and coefficient results of linear regression, together with congruity between newly gained and previously reported lists.

SNPs for constructing genetic risk score (GRS) were sorted out by removing the other SNPs in linkage disequilibrium (LD) with D' greater than 0.80. A weighted GRS was developed (as the second IV, indicated as GRS1 in the later sections) using these mutually independent SNPs, with each variant's effect size according to the number of risk alleles. For each genotype result of selected SNPs, participants received an allele score of 0, 1, or 2 for carrying zero (wild-type homozygous), one (heterozygous), or two (homozygous for the risk allele) adiponectin-decreasing or BMI-increasing risk alleles, respectively. The GRS was then calculated by adding scores of all selected SNPs, weighted by the respective coefficient for one or two allele change estimated for the corresponding SNP.

6. Definitions of outcomes

The outcome variable for this research was serum aminotransferase level, defined in two ways. The first one was a binary outcome, which included elevated and normal aminotransferase levels. A problematic outcome was considered as a group of people who had AST ≥ 40 IU/L or ALT ≥ 40 IU/L. The second was a continuous level outcome of serum ALT, a highly specific liver enzyme recognized as a more appropriate biomarker for liver injury.

Participants who demonstrated extremely high AST or ALT levels that exceeded three times the upper normal limit (>120 IU/L) were excluded from the study, because it was highly suggestive of liver disease with distinct etiology other than or independent of obesity.

7. Sensitivity analyses

To increase reliability of the study results, another GRS was generated (as the third IV, indicated as GRS2 in the later sections) using candidate SNPs for each trait, adiponectin level and BMI. Among diverse loci which have been revealed to affect adiponectin level, *ADIPOQ* variants were selected as the candidate SNPs for constructing adiponectin GRS. Similarly, SNPs in *FTO* or *MC4R* genes were chosen for the BMI GRS. SNPs were selected according to the order of their significance level, and all other interrelated SNPs with LD were excluded. Methods for GRS calculation were similarly conducted as those described above.

Another sensitivity analysis was conducted after excluding heavy drinkers because they could have elevated liver enzyme levels due to exogenous effect. Among 3,531 participants who had the information of alcohol drinking amount, those with an average intake of more than 50g per day (n=263) were excluded. In the analysis, the amount of alcohol drinking was used as a covariate for adjustment.

Potential pleiotropy and genetic confounders were also assessed. First, although limitedly, extra route from genetic instruments (G) to the outcome variable (Y), other than exposure variable (X) itself, was evaluated; it was done graphically by checking if any alteration in effect on outcome estimates (Y) was observed within the independent genetic instruments (G) of respectively different effect sizes for their related phenotype. Second, reciprocal

relationships between exposure and outcome variables were explored using genetic traits determining the liver enzyme levels; in order to assess further existence of genetic variants that were related to both exposure (X) and outcome (Y) variables. The LD relationships between SNPs determining liver enzyme levels (Y) and SNPs used as genetic instruments (G) of adiponectin level and BMI (X) were also evaluated. Third, SNPs used in the IV of adiponectin levels or BMI (X), respectively, were tested if they had any LD association with each other in the same chromosome.

8. Statistical analysis

In order to assess general characteristics of study participants, descriptive analysis was conducted; means and standard deviations (SD) were calculated for continuous variables, and frequencies and relative proportions were estimated for categorical variables. All of measured variables that showed skewed distribution were log-transformed for the statistical tests and regression analyses.

In performing GWAS, associations of each SNP with serum adiponectin level and BMI were tested by linear regression under an additive genetic model adjusting for age and sex. It was performed using PLINK 1.07 (http://pngu.mgh.harvard.edu/~purcell/plink/). Haploview version 4.1 (http://www.broad.mit.edu/mpg/haploview/) was used to generate Manhattan plots and evaluate LD structures. With an adjustment for age and sex, per

allele changes and effects of different genotypes on adiponectin level and BMI were calculated by ordinary least square (OLS) regression, using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). Evaluation for the liver enzyme level-related SNPs were conducted with identical methods and tools with additional adjusting for alcohol consumption status.

Tests for the differences in variables among the three genotypes for each representative SNP were performed by ANOVA and chi-square tests. In case of continuous variables, associations between these potential confounders and GRS were evaluated through correlation analysis. For categorical variables, nonparametric test such as Wilcoxon rank two-sample or Kruskal-Wallis test was used for adiponectin GRS, and chi-square test was used in BMI GRS. To evaluate observed estimates between an exposure of interest and outcomes, OLS regression or conventional multivariate logistic regression method was used.

Mendelian randomization analysis was performed with two different methods: Wald estimator and two-stage least squares method. The first common step in both methods was to assess the association between genetic traits and exposure variables. Linear regression was used to measure the strength of the association between genetic variants or GRS and the exposures of interest (X), under the assumption of an additive genetic model. The significance of genetic variables was estimated by F statistics, and the final

regression coefficients of the genetic IV (G) on the exposure variables (X) is expressed as $\hat{\beta}_{XG}$ in the description below.

Using this result, we assessed the causal effect of X on binary outcome Y using the Wald estimator approach. The final estimates, or the log odds ratios of abnormal AST or ALT per unit decrease in adiponectin and increase in BMI, respectively, were calculated using a Wald/ratio estimator [42]:

$$\hat{\beta}_{IV} = \frac{\log(OR_{YG})}{\hat{\beta}_{XG}}$$

The confidence interval for this ratio estimate was obtained using the delta method, which is also termed as a Taylor series expansion.[40]

We also assessed the causal effect of X on continuous outcome Y using the two-stage least squares (2SLS) approach. It consists of two stages: (1) A conventional least-squares regression between the genetic IV (G) and the exposure (X) variables (2) Subsequent least-squares regression of the outcome Y on the predicted value of exposure (X), which could be derived from the first stage regression and saved as $\widehat{X}$. Such an approach can be simplified as follows:

$$y_{1i} = \beta_0 + \beta_1 \hat{y}_{2i} + \beta_2 z_i + u_i \quad (2)$$

$$\hat{y}_{2i} = \widehat{X}_i = \pi_0 + \pi_1 z_i + \pi_2 g_i + v_i \quad (1)$$

Here, y_{1i} is the outcome variable (Y); $\hat{y}_{2i}$, the endogenous regressor, is the predicted exposure (X) calculated from the results of the first regression (π_2 would be substituted with $\hat{\beta}_{XG}$ when g_i is a genetic IV); z_i and g_i represent the exogenous regressors, collectively referred as the instruments; u_i and v_i are error terms for the i th observation.

To examine whether the genetically predicted exposure variables ($\hat{y}_{2i}$) were endogenous in reality, we performed a Durbin and Wu-Hausman test that assesses covariance between exposure variable of interest and residuals. These Mendelian randomization analyses were performed with the STATA package *ivregress*, using STATA software version 12 (Stata Corp. LP, College Station, TX, USA). All statistical tests were two-sided and p <0.05 was considered statistically significant.

9. Ethics statement

This study was approved by an Institutional Review Board of Graduate school of Public Health, Yonsei University [IRB Number: 2-1040939-AB-N-01-2016-111].

III. Results

1. General characteristics of study population

A total of 3,793 participants (2,483 men and 1,310 women) were included in the analysis from the KCPS-II Biobank subcohort, with the measurement of adiponectin and BMI levels as well as the genotyping of relevant genomes. General characteristics of the study participants are summarized in Table 1. Participants were primarily early middle-aged, and the distribution of key variables except age showed statistically significant difference between men and women. A large proportion of non-drinkers and non-smokers were women, whereas the most of alcohol drinkers and smokers were men.

Table 1. General characteristics of the study population, by sex

		Total (N=3,793)		Men (N=2,483)		Women (N=1,310)	
		Mean/N	(SD,%)	Mean/N	(SD,%)	Mean/N	(SD,%)
Age (years)		42.55	(8.8)	42.47	(8.6)	42.69	(9.3)
BMI (kg/m^2)		23.73	(3.0)	24.46	(2.7)	22.34	(3.1)
Waist circumference (cm)		81.16	(9.0)	84.77	(7.2)	74.33	(8.1)
Adiponectin (μg/mL)		7.13	(4.1)	5.93	(3.1)	9.41	(4.8)
Bilirubin (mg/mL)		0.92	(0.4)	0.99	(0.4)	0.78	(0.3)
Albumin (g/mL)		4.57	(0.2)	4.62	(0.2)	4.50	(0.2)
Education (years)		14.48	(3.0)	15.18	(2.6)	13.44	(3.3)
Drinking	**No**	890	(23.5)	261	(10.5)	629	(48.0)
	Yes	2903	(76.5)	2222	(89.5)	681	(52.0)
Smoking	**No**	1853	(48.9)	647	(26.1)	1206	(92.1)
	Past	767	(20.2)	706	(28.4)	61	(4.7)
	Current	1173	(30.9)	1130	(45.5)	43	(3.3)
Exercise	**No**	2297	(62.9)	1617	(67.0)	680	(54.8)
	Yes	1357	(37.1)	797	(33.0)	560	(45.2)

2. SNPs associated with exposure variables

A genome-wide association with adiponectin level and BMI in 3,793 individuals revealed a number of genetic polymorphisms affecting these intermediate phenotypes. Tables 2 and 3 show the list of 20 SNPs relevant to serum adiponectin level and BMI, respectively, with the lowest *p*-values in the study participants. Manhattan plots of these genome-wide analyses are provided in Appendix B1.

Most of the highly significant polymorphisms for adiponectin level belonged to chromosome 16, which includes the *CDH13* gene, and majority of those SNPs were located on that gene or were in LD with SNPs on that gene. Similarly, but less markedly, the highly significant SNPs associated with BMI were relatively concentrated on chromosome 11, which includes the *BDNF* gene. Most of those SNPs were related to each other as in LD. Finally, it was rs4783244 and rs2030323 variants that were selected as a single SNP instrumental variable for Mendelian randomization analysis, respectively. Weighted GRSs were also created using 7 representative SNPs for adiponectin level and 13 representative SNPs for body mass index.

Most of SNPs with low p-values that were located in or nearby *CDH13* gene revealed high significance for adiponectin levels ($p = 9.30 \times 10^{-44}$ to 4.24×10^{-16}), whereas the

significance of SNPs located in or nearby *BDNF* gene were not much strong (p = 3.14×10^{-7} to 1.02×10^{-5}) for their relationships with BMI.

Table 2. Top 20 SNPs strongly relevant to serum adiponectin level among the study population, without hepatitis B or C viral infection

Chr	SNP	Risk allele	Other allele	RAF	Nearest gene	per allele change		1 Risk allele		2 Risk alleles		GRS1
						beta	SE	beta	SE	beta	SE	
16	rs16957889	G	A	0.300	*CDH13*	-0.1796	(0.0139)	-0.1620	(0.0190)	-0.3820	(0.0326)	
16	rs4783244	T	G	0.301	*CDH13*	-0.1805	(0.0140)	-0.1658	(0.0191)	-0.3802	(0.0327)	
16	rs12596316	G	A	0.306	*CDH13*	-0.1721	(0.0139)	-0.1570	(0.0191)	-0.3630	(0.0322)	
16	rs3852724	A	C	0.543	*CDH13*	-0.1072	(0.0131)	-0.0934	(0.0241)	-0.2121	(0.0264)	*
16	rs7193788	G	A	0.453	*CDH13*	-0.1057	(0.0133)	-0.0764	(0.0213)	-0.2176	(0.0267)	*
16	rs16957913	C	T	0.221	*CDH13*	-0.1182	(0.0157)	-0.1101	(0.0196)	-0.2570	(0.0435)	*
3	rs864265	T	G	0.091	*ADIPOQ*	-0.1494	(0.0229)	-0.1609	(0.0246)	-0.1640	(0.1144)	*
3	rs74577862	A	G	0.019	*ADIPOQ*	-0.3153	(0.0472)	-0.3128	(0.0493)	-0.6859	(0.3226)	
3	rs2036373	G	T	0.037	*ADIPOQ*	-0.2149	(0.0355)	-0.2149	(0.0355)	NA		*
16	rs3865183	C	T	0.584	*CDH13*	-0.0744	(0.0133)	-0.0512	(0.0261)	-0.1417	(0.0275)	
16	rs3852728	G	A	0.837	*CDH13*	-0.0863	(0.0176)	-0.0991	(0.0580)	-0.1828	(0.0563)	
16	rs17244777	C	A	0.228	*CDH13*	-0.0639	(0.0157)	-0.0594	(0.0195)	-0.1395	(0.0437)	*
8	rs28455997	C	T	0.064	*CSMD1*	-0.1130	(0.0267)	-0.1106	(0.0285)	-0.2588	(0.1451)	*
16	rs8062678	T	G	0.829	*CDH13*	-0.0719	(0.0174)	-0.0911	(0.0565)	-0.1589	(0.0549)	
8	rs10091165	A	C	0.066	*CSMD1*	-0.1053	(0.0261)	-0.1059	(0.0283)	-0.2038	(0.1292)	
8	rs73185530	A	G	0.065	*CSMD1*	-0.1041	(0.0264)	-0.1024	(0.0283)	-0.2286	(0.1364)	
8	rs17079690	C	G	0.073	*CSMD1*	-0.1041	(0.0249)	-0.0813	(0.0270)	-0.4456	(0.1199)	
8	rs74350387	C	A	0.067	*CSMD1*	-0.1063	(0.0261)	-0.1049	(0.0281)	-0.2293	(0.1326)	
8	rs117585453	A	G	0.066	*CSMD1*	-0.1035	(0.0261)	-0.1017	(0.0281)	-0.2285	(0.1326)	
16	rs79003691	T	C	0.035	*CDH13*	-0.1620	(0.0351)	-0.1508	(0.0374)	-0.4877	(0.1984)	

Adjusted for age and sex

SNP, single nucleotide polymorphism; RAF, Risk allele frequency

* SNPs used for GRS1: selected SNPs after excluding highly interrelated SNPs with linkage disequilibrium (LD) from the original top 20 SNPs

Table 3. Top 20 SNPs strongly relevant to body mass index among the study population, without hepatitis B or C viral infection

Chr	SNP	Risk allele	Other allele	RAF	Nearest gene	per allele change		1 Risk allele		2 Risk alleles		GRS1
						beta	SE	beta	SE	beta	SE	
11	rs2030323	C	A	0.545	*BDNF*	0.3068	(0.0700)	0.2987	(0.1290)	0.6123	(0.1413)	*
11	rs6265	C	T	0.538	*BDNF*	0.2954	(0.0699)	0.2807	(0.1272)	0.5886	(0.1407)	
11	rs11230563	T	C	0.197	*CD6*	0.3899	(0.0874)	0.3758	(0.1058)	0.8254	(0.2607)	*
11	rs11030084	C	T	0.550	*BDNF-AS*	0.2700	(0.0699)	0.1043	(0.1299)	0.5084	(0.1413)	
8	rs2890502	T	C	0.928	*EYA1*	0.5911	(0.1332)	0.2098	(0.6418)	0.8357	(0.6296)	*
11	rs4074134	C	T	0.546	*BDNF-AS*	0.2616	(0.0699)	0.1139	(0.1290)	0.4972	(0.1411)	
11	rs10501087	T	C	0.538	*BDNF-AS*	0.2585	(0.0698)	0.1685	(0.1274)	0.5034	(0.1406)	
8	rs16875189	T	C	0.088	*ABRA*	0.4501	(0.1233)	0.4769	(0.1329)	0.6024	(0.6054)	*
5	rs56718608	A	C	0.082	*C5orf66*	0.5706	(0.1255)	0.5648	(0.1374)	1.1944	(0.5708)	*
9	rs10858232	A	G	0.166	*LHX3*	0.3762	(0.0929)	0.4402	(0.1099)	0.4963	(0.2997)	*
18	rs17782313	C	T	0.248	*MC4R*	0.3354	(0.0792)	0.3535	(0.1038)	0.6373	(0.2016)	*
18	rs571312	A	C	0.247	*MC4R*	0.3317	(0.0795)	0.3635	(0.1036)	0.6023	(0.2038)	
2	rs79091937	G	A	0.889	*SEPT2*	0.4067	(0.1110)	0.6409	(0.4626)	1.0191	(0.4524)	*
14	rs139904021	C	T	0.978	*PSMA3*	1.0828	(0.2486)	reference		1.0828	(0.2486)	*
4	rs6844085	T	G	0.941	*FIP1L1*	0.5186	(0.1502)	0.5320	(0.8830)	1.0498	(0.8717)	*
18	rs10871777	G	A	0.248	*MC4R*	0.3300	(0.0792)	0.3567	(0.1039)	0.6106	(0.2009)	
8	rs6986205	G	A	0.011	*OXR1*	1.3629	(0.3425)	1.3629	(0.3425)	NA		*
7	rs140874255	C	T	0.982	*ZNF890P*	1.1407	(0.2698)	reference		1.1407	(0.2698)	*
7	rs80171753	T	C	0.979	*ZNRF2*	1.0589	(0.2558)	reference		1.0589	(0.2558)	*
18	rs1350341	A	G	0.286	*MC4R*	0.3272	(0.0766)	0.3401	(0.1028)	0.6352	(0.1846)	

Adjusted for age and sex

SNP, single nucleotide polymorphism; RAF, Risk allele frequency

* SNPs used for GRS1: selected SNPs after excluding highly interrelated SNPs with linkage disequilibrium (LD) from the original top 20 SNPs

Candidate SNPs of the target genes selected by GWAS analysis are listed in Tables 4 and 5. They showed relatively weak significances in association with their phenotypes. Hence, the entire independent SNPs detected for each trait were collectively used to construct GRS. A total of 6 SNPs in *ADIPOQ* gene, 8 SNPs in *FTO* gene, and 5 SNPs in *MC4R* genes were finally selected.

Table 4. A list of 6 SNPs for construction of candidate genetic risk score (GRS2) for adiponectin level

Chr	SNP	Risk allele	Other allele	RAF	Gene	per allele change		1 Risk allele		2 Risk alleles	
						beta	SE	beta	SE	beta	SE
3	rs864265	T	G	0.091	*ADIPOQ*	-0.1494	(0.0229)	-0.1609	(0.0246)	-0.1640	(0.1144)
3	rs2036373	G	T	0.037	*ADIPOQ*	-0.2149	(0.0355)	-0.2149	(0.0355)	NA	
3	rs2117986	C	T	0.269	*ADIPOQ*	-0.0662	(0.0147)	-0.0719	(0.0194)	-0.1225	(0.0366)
3	rs117147558	T	C	0.077	*ADIPOQ*	-0.0286	(0.0246)	-0.0261	(0.0265)	-0.0862	(0.1231)
3	rs17366568	A	G	0.022	*ADIPOQ*	-0.0868	(0.0452)	-0.0868	(0.0452)	NA	
3	rs1501299	T	G	0.303	*ADIPOQ*	-0.0115	(0.0142)	-0.0176	(0.0195)	-0.0154	(0.0329)

Adjusted for age and sex

SNP, single nucleotide polymorphism; RAF, Risk allele frequency

Table 5. A list of 13 SNPs for construction of candidate genetic risk score (GRS2) for body mass index

Chr	SNP	Risk allele	Other allele	RAF	Gene	per allele change		1 Risk allele		2 Risk alleles	
						beta	SE	beta	SE	beta	SE
16	rs9302654	T	C	0.132	*FTO*	0.3459	(0.1033)	0.2973	(0.1158)	1.0132	(0.4034)
16	rs116978290	G	A	0.960	*FTO*	0.5693	(0.1890)	1.8511	(1.7520)	2.3906	(1.7428)
16	rs879679	C	T	0.239	*FTO*	0.2304	(0.0807)	0.2188	(0.1038)	0.4851	(0.2112)
16	rs76368010	C	T	0.923	*FTO*	0.1964	(0.1303)	0.8078	(0.6564)	0.9541	(0.6456)
16	rs12446738	A	C	0.013	*FTO*	0.8015	(0.3134)	0.8015	(0.3134)	NA	
16	rs2058908	C	T	0.700	*FTO*	0.1411	(0.0758)	0.2222	(0.1813)	0.3288	(0.1788)
16	rs117659448	C	T	0.024	*FTO*	0.4467	(0.2285)	0.4467	(0.2285)	NA	
16	rs7185479	C	A	0.157	*FTO*	0.1765	(0.0953)	0.1457	(0.1114)	0.4901	(0.3199)
18	rs17782313	C	T	0.248	*MC4R*	0.3354	(0.0792)	0.3535	(0.1038)	0.6373	(0.2016)
18	rs55963627	T	G	0.042	*MC4R*	0.2097	(0.1722)	0.1588	(0.1811)	1.3378	(1.0679)
18	rs187398163	A	G	0.984	*MC4R*	0.4413	(0.2933)	reference		0.4413	(0.2933)
18	rs2229616	C	T	0.976	*MC4R*	0.2441	(0.2324)	reference		0.2441	(0.2324)
18	rs7239577	T	C	0.031	*MC4R*	0.2295	(0.2031)	0.2295	(0.2031)	NA	

Adjusted for age and sex

SNP, single nucleotide polymorphism; RAF, Risk allele frequency

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

Genotype frequencies of adiponectin-related rs4783244 for genotypes GG, TG, and TT were 48.4%, 42.1%, and 9.0%, respectively, in the study population. Mean adiponectin level was the lowest in rs4783244*TT carriers (5.42 ± 3.23 mg/dL, n=343), and increased gradually as the number of risk allele (T) declined. Genotype frequencies of BMI-related rs2030323 for genotypes CC, AC, and AA were 29.5%, 50.0%, and 20.2%, respectively. Mean BMI was the highest in rs2030323 homozygous carriers of risk allele (CC) with mean BMI of 24.01 kg/m^2 (SD 3.01 kg/m^2, n=1,119), and decreased as C allele declined (Tables 6 and 7). Figure 3 describes a distribution of GRS1 for adiponectin levels and BMI which consist of de novo SNPs marked with corresponding mean levels of adiponectin and BMI, respectively.

To assess the second assumption of Mendelian randomization analysis described in the methods section, we tested whether single SNPs or GRSs used as the IV were associated with potential confounders (Tables 6 and 7). No significant differences across genotype groups and GRS were shown regarding age, sex, drinking status, and exercise status, for both intermediate phenotype variables of adiponectin and BMI. Genetic traits for adiponectin level had no association with BMI, waist circumference, and education level; however they showed significant association with smoking status among the genotype subgroups. Genetic traits for BMI were significantly associated with waist circumference,

adiponectin level (in case of GRS), and education level (among the genotype subgroups), but had no relationship with smoking status.

Table 6. Associations of potential confounders with *CDH13* polymorphism at rs4783244 for adiponectin level and genetic risk scores among the study population

rs4783244		GG (N=1,837)	TG (N=1,597)	TT (N=343)		GRS1[a]		GRS2[b]	
Variables		Mean/N (SD,%)	Mean/N (SD,%)	Mean/N (SD,%)	*p*	r/mean (SD)	*p*	r/mean (SD)	*p*
Age		42.39 (8.74)	42.58 (8.93)	43.22 (8.39)	*0.266*	0.027	*0.105*	0.001	*0.973*
Sex	**Men**	1202 (48.66)	1028 (41.62)	240 (9.72)		4.56 (3.6)		1.12 (1.3)	
	Women	635 (48.58)	569 (43.53)	103 (7.88)	*0.141*	4.57 (3.5)	*0.608* [d]	1.07 (1.3)	*0.055* [d]
BMI		23.65 (3.00)	23.8 (3.02)	23.79 (3.10)	*0.335*	0.008	*0.613*	0.012	*0.448*
Waist circumference		80.87 (8.92)	81.37 (9.19)	81.69 (8.98)	*0.139*	0.008	*0.619*	0.0002	*0.989*
Adiponectin[c]		7.85 (4.34)	6.66 (3.76)	5.42 (3.23)	*<0.001* [c]	-0.208 [c]	*<0.001* [c]	-0.143 [c]	*<0.001* [c]
Education		14.48 (2.95)	14.44 (3.07)	14.71 (2.71)	*0.613*	0.013	*0.614*	0.004	*0.871*
Drinking	**No**	418 (47.13)	385 (43.40)	84 (9.47)		4.68 (3.6)		1.09 (1.3)	
	Yes	1419 (49.10)	1212 (41.94)	259 (8.96)	*0.582*	4.52 (3.6)	*0.143* [d]	1.10 (1.3)	*0.331* [d]
Smoking	**No**	876 (47.38)	813 (43.97)	160 (8.65)		4.62 (3.6)		1.11 (1.3)	
	Past	365 (47.96)	305 (40.08)	91 (11.96)		4.66 (3.7)		1.14 (1.3)	
	Current	596 (51.07)	479 (41.05)	92 (7.88)	*0.008*	4.39 (3.5)	*0.135* [d]	1.05 (1.2)	*0.560* [d]
Exercise	**No**	1082 (47.33)	982 (42.96)	222 (9.71)		4.64 (3.6)		1.11 (1.3)	
	Yes	683 (50.52)	559 (41.35)	110 (8.14)	*0.100*	4.42 (3.5)	*0.086* [d]	1.09 (1.3)	*0.948* [d]

[a] GRS1: Using de novo SNPs obtained from GWAS

[b] GRS2: Using candidate SNPs from the target gene (*ADIPOQ*)

[c] Tests for difference performed with log-transformed value

[d] Tests for difference conducted by nonparametric methods

Table 7. Associations of potential confounders with *BDNF* polymorphism at rs2030323 for body mass index and genetic risk scores among the study population

rs2030323		CC (N=1,119)	AC (N=1,896)	AA (N=765)		GRS1[a]		GRS2[b]	
Variables		Mean/N (SD,%)	Mean/N (SD,%)	Mean/N (SD,%)	*p*	r/mean (SD)	*p*	r/mean (SD)	*p*
Age		42.82 (8.88)	42.37 (8.72)	42.59 (8.95)	*0.396*	0.017	*0.296*	-0.001	*0.947*
Sex	**Men**	712 (28.78)	1256 (50.77)	506 (20.45)		9.56 (1.3)		8.00 (1.1)	
	Women	407 (31.16)	640 (49.00)	259 (19.83)	*0.311*	9.60 (1.3)	*0.455*	7.94 (1.1)	*0.177*
BMI		24.01 (3.01)	23.70 (3.01)	23.4 (3.03)	*<0.001*	0.216	*<0.001*	0.108	*<0.001*
Waist circumference		81.6 (8.98)	81.18 (8.94)	80.53 (9.37)	*0.039*	0.161	*<0.001*	0.084	*<0.001*
Adiponectin[c]		7.06 (4.24)	7.12 (4.00)	7.23 (4.04)	*0.510* [c]	-0.038 [c]	*0.021* [c]	-0.015 [c]	*0.377* [c]
Education		14.23 (3.13)	14.73 (2.77)	14.21 (3.22)	*0.003*	-0.032	*0.197*	0.007	*0.794*
Drinking	**No**	272 (30.63)	418 (47.07)	198 (22.30)		9.56 (1.3)		7.95 (1.1)	
	Yes	847 (29.29)	1478 (51.11)	567 (19.61)	*0.080*	9.58 (1.3)	*0.690*	7.99 (1.1)	*0.330*
Smoking	**No**	553 (29.96)	931 (50.43)	362 (19.61)		9.57 (1.3)		7.96 (1.1)	
	Past	222 (28.98)	386 (50.39)	158 (20.63)		9.54 (1.3)		8.02 (1.1)	
	Current	344 (29.45)	579 (49.57)	245 (20.98)	*0.905*	9.61 (1.3)	*0.553*	7.98 (1.1)	*0.389*
Exercise	**No**	675 (29.50)	1144 (50.00)	469 (20.50)		9.58 (1.3)		7.99 (1.1)	
	Yes	400 (29.56)	691 (51.07)	262 (19.36)	*0.691*	9.56 (1.3)	*0.678*	7.95 (1.1)	*0.226*

[a] GRS1: Using de novo SNPs obtained from GWAS [b] GRS2: Using candidate SNPs from the target genes (*FTO* and *MC4R*)

[c] Tests for difference performed with log-transformed value

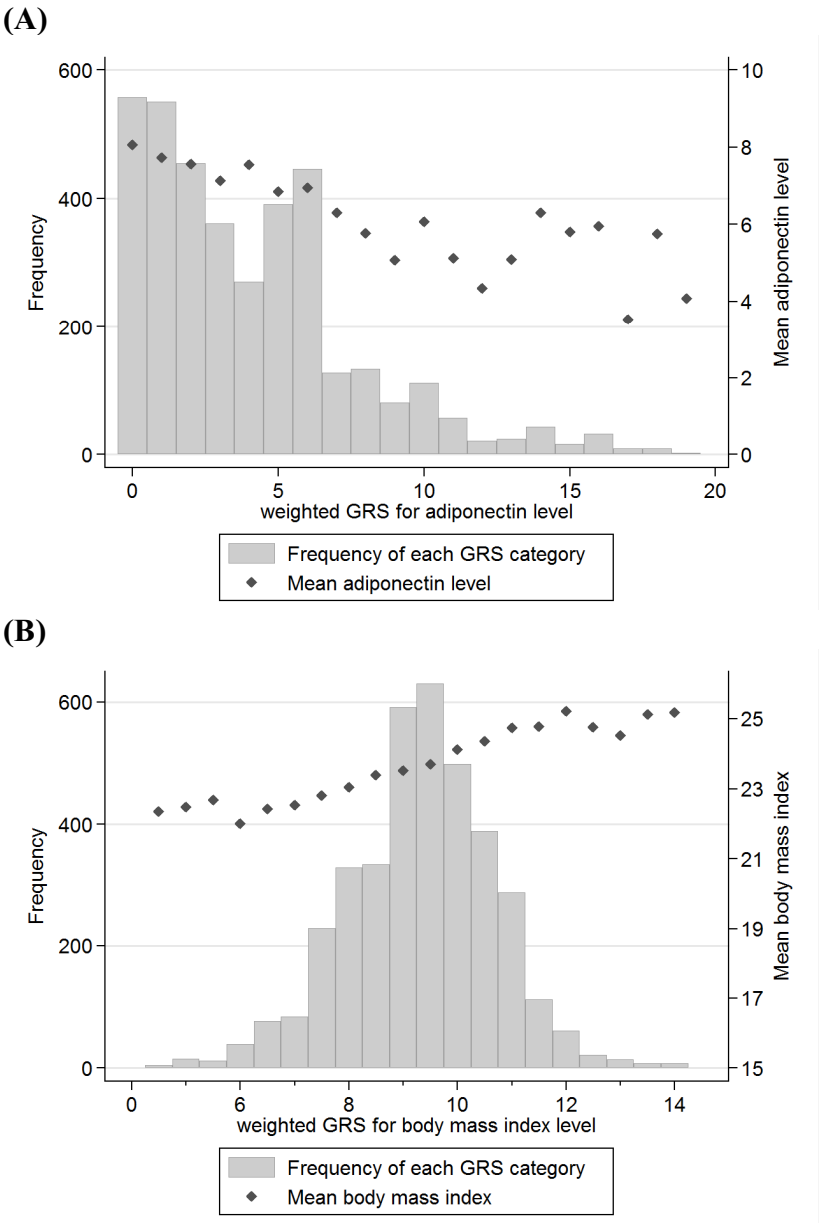

Figure 3. (A) Distribution of weighted GRS for adiponectin level and corresponding mean adiponectin levels; (B) Distribution of weighted GRS for body mass index and corresponding mean body mass index

4. Results of conventional regression and Mendelian randomization analyses

Using the highly associated SNPs and GRSs from de novo SNPs, we conducted Mendelian randomization analysis to investigate whether adiponectin level and BMI cause elevation of liver enzymes; we compared these results to the results from conventional observational analyses (Tables 8-11). The first-stage F-statistics for the association between genetic traits and adiponectin levels or BMI were high enough to perform the analyses.

For the analyses regarding adiponectin levels, it seemed to significantly decrease both the risk of elevation in liver aminotransferase (AST or ALT) levels (Table 8 and Figure 4) and log-transformed ALT level (Table 9), in conventional regression analyses. However, these relationships were not significant in Mendelian randomization analysis using the IV method, with any kind of genetic IV being used (Tables 8, 9 and Figure 4).

As for BMI, the results of conventional regression analyses regarding liver enzyme levels were also significant, for both binary (Table 10 and Figure 5) and continuous (Table 11) outcomes. With Mendelian randomization using GRS1, these positive associations maintained the significance for the binary outcome (OR=1.36 with 95% CI from 1.16 to 1.59, adjusted for age and sex) and the continuous outcome (β = 0.051 with p<0.001,

adjusted for age and sex), implying that a causal relationship between BMI and the elevation in liver enzyme levels was reasonable (Tables 10, 11 and Figure 5).

Durbin-Wu–Hausman endogeneity test for adiponectin and BMI levels, respectively, revealed *p*-values of clear or borderline significance (results not shown) to reject the null hypothesis (H_0: instrumented regressors are in fact exogenous), thereby supporting the use of Mendelian randomization or IV method.

Table 8. Association between adiponectin and abnormal liver enzyme level[§], among subjects without hepatitis B or C viral infection

Explanatory variables	Outcome variable	Instrumental variable		Change in log-transformed adiponectin per increase of one risk allele or one unit of GRS					Observational multivariate logistic regression analysis			Mendelian randomization analysis	
				β	SE	F	R^2	*p*	OR	CI	*p*	OR	CI
Adiponectin[c]	elevated serum AST or ALT	1 SNP (rs4783244)	Model 1	-0.180	0.014	167	0.043	<.001	0.36	(0.30-0.43)	<.001	1.10	(0.50-2.45)
			Model 2	-0.177	0.013	288	0.189	<.001	0.48	(0.40-0.58)	<.001	1.17	(0.51-2.68)
			Model 3	-0.176	0.013	119	0.189	<.001	0.48	(0.40-0.58)	<.001	1.19	(0.51-2.78)
		GRS1[a]	Model 1	-0.033	0.003	162	0.043	<.001	0.36	(0.30-0.43)	<.001	0.91	(0.41-2.03)
			Model 2	-0.033	0.002	285	0.191	<.001	0.48	(0.40-0.58)	<.001	0.86	(0.38-1.93)
			Model 3	-0.033	0.002	118	0.191	<.001	0.48	(0.40-0.58)	<.001	0.86	(0.38-1.96)
		GRS2[b] (*ADIPOQ*)	Model 1	-0.063	0.007	75.8	0.020	<.001	0.36	(0.30-0.43)	<.001	0.73	(0.23-2.33)
			Model 2	-0.060	0.007	243	0.167	<.001	0.48	(0.40-0.58)	<.001	0.82	(0.23-2.86)
			Model 3	-0.060	0.007	101	0.168	<.001	0.48	(0.40-0.58)	<.001	0.84	(0.23-3.00)

[a] GRS1: Using de novo 7 SNPs obtained from GWAS [b] GRS2: Using 6 candidate SNPs from the target gene (*ADIPOQ*)

[c] Log-transformed

[§] Binary outcome defined as serum aminotransferase (AST; Aspartate aminotransferase, or ALT; Alanine transaminase) level ≥ 40IU/L

Model 1: Crude model

Model 2: Adjusted for age and sex

Model 3: Adjusted for age, sex, smoking status, alcohol drinking, and exercise status

Table 9. Association between adiponectin and serum ALT[§] level, among subjects without hepatitis B or C viral infection

Explanatory variables	Outcome variable	Instrumental variable		Change in log-transformed adiponectin per increase of one risk allele or one unit of GRS					Observational multivariate linear regression analysis			Mendelian randomization analysis		
				β	SE	F	R^2	*p*	β	SE	*p*	β_{IV}	SE	*p*
Adiponectin[c]	serum ALT level[c]	1 SNP (rs4783244)	Model 1	-0.180	0.014	167	0.043	<.001	-0.292	0.015	<.001	-0.038	0.073	0.609
			Model 2	-0.177	0.013	288	0.189	<.001	-0.139	0.015	<.001	-0.009	0.067	0.892
			Model 3	-0.176	0.013	119	0.189	<.001	-0.138	0.015	<.001	-0.001	0.069	0.990
		GRS1[a]	Model 1	-0.033	0.003	162	0.043	<.001	-0.292	0.015	<.001	-0.019	0.075	0.798
			Model 2	-0.033	0.002	285	0.191	<.001	-0.139	0.015	<.001	-0.028	0.066	0.665
			Model 3	-0.033	0.002	118	0.191	<.001	-0.138	0.015	<.001	-0.028	0.068	0.677
		GRS2[b] (*ADIPOQ*)	Model 1	-0.063	0.007	75.8	0.020	<.001	-0.292	0.015	<.001	-0.051	0.114	0.656
			Model 2	-0.060	0.007	243	0.167	<.001	-0.139	0.015	<.001	0.002	0.106	0.986
			Model 3	-0.060	0.007	101	0.168	<.001	-0.138	0.015	<.001	-0.010	0.108	0.925

[a] GRS1: Using de novo 7 SNPs obtained from GWAS [b] GRS2: Using 6 candidate SNPs from the target gene (*ADIPOQ*)
[c] Log-transformed [§] ALT, Alanine transaminase (continuous outcome)

Model 1: Crude model
Model 2: Adjusted for age and sex
Model 3: Adjusted for age, sex, smoking status, alcohol drinking, and exercise status

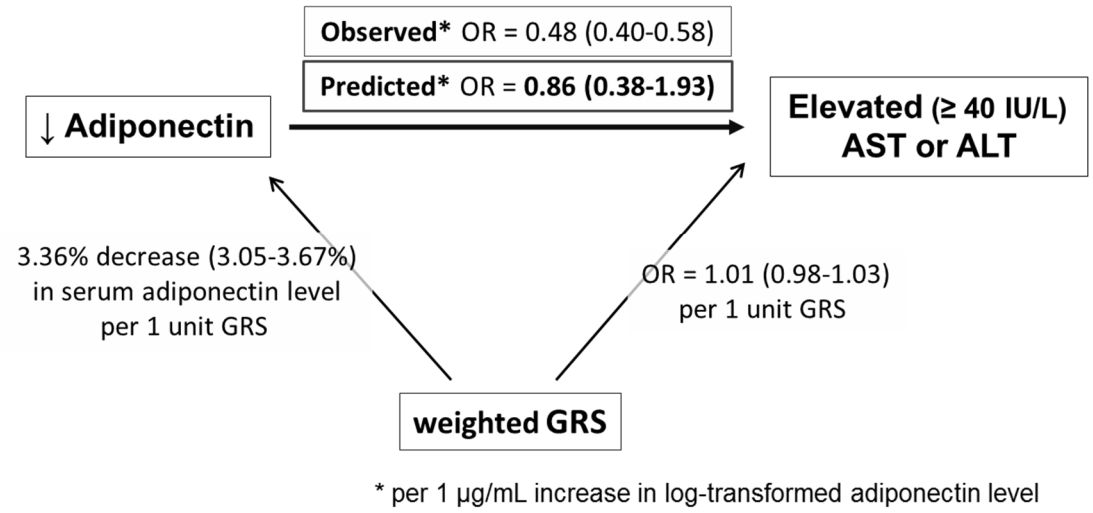

Figure 4. Mendelian randomization of serum adiponectin level and elevated serum aminotransferase levels (AST, Aspartate aminotransferase; ALT, Alanine transaminase)

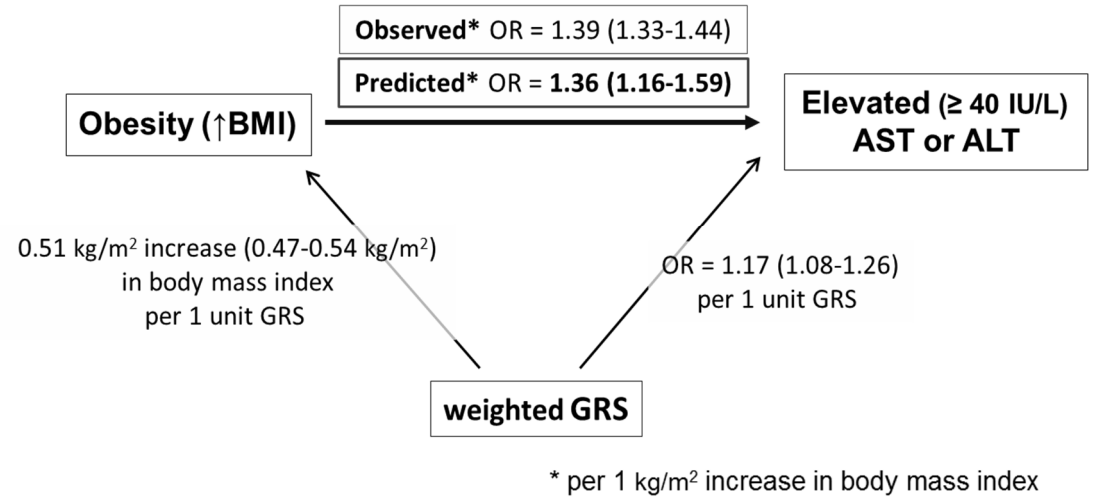

Figure 5. Mendelian randomization of body mass index and elevated serum aminotransferase levels (AST, Aspartate aminotransferase; ALT, Alanine transaminase)

Table 10. Association between body mass index and abnormal liver enzyme level[§], among subjects without hepatitis B or C viral infection

Explanatory variables	Outcome variable	Instrumental variable		Change in body mass index per increase of one risk allele or one unit of GRS					Observational multivariate logistic regression analysis			Mendelian randomization analysis	
				β	SE	F	R^2	*p*	OR	CI	*p*	OR	CI
BMI	elevated serum AST or ALT	1 SNP (rs2030323)	Model 1	0.307	0.070	19.2	0.005	<.001	1.41	(1.36-1.47)	<.001	1.12	(0.72-1.73)
			Model 2	0.330	0.065	198	0.136	<.001	1.39	(1.33-1.44)	<.001	1.19	(0.78-1.81)
			Model 3	0.313	0.066	88.3	0.144	<.001	1.40	(1.34-1.45)	<.001	1.18	(0.85-1.63)
		GRS1[a]	Model 1	0.502	0.037	182	0.046	<.001	1.41	(1.36-1.47)	<.001	1.32	(1.14-1.54)
			Model 2	0.506	0.035	270	0.178	<.001	1.39	(1.33-1.44)	<.001	1.36	(1.16-1.59)
			Model 3	0.487	0.035	116	0.184	<.001	1.40	(1.34-1.45)	<.001	1.36	(1.16-1.61)
		GRS2[b] (*FTO/MC4R*)	Model 1	0.295	0.045	43.2	0.011	<.001	1.41	(1.36-1.47)	<.001	1.22	(0.90-1.66)
			Model 2	0.275	0.042	202	0.141	<.001	1.39	(1.33-1.44)	<.001	1.18	(0.85-1.65)
			Model 3	0.264	0.042	89.3	0.149	<.001	1.40	(1.34-1.45)	<.001	1.18	(0.83-1.67)

[a] GRS1: Using de novo 13 SNPs obtained from GWAS [b] GRS2: Using 13 candidate SNPs from the target genes (*FTO* and *MC4R*)

[§] Binary outcome defined as serum aminotransferase (AST; Aspartate aminotransferase, or ALT; Alanine transaminase) level ≥ 40IU/L

Model 1: Crude model

Model 2: Adjusted for age and sex

Model 3: Adjusted for age, sex, smoking status, alcohol drinking, and exercise status

Table 11. Association between body mass index and serum ALT[§] level, among subjects without hepatitis B or C viral infection

Explanatory variables	Outcome variable	Instrumental variable		Change in body mass index per increase of one risk allele or one unit of GRS					Observational multivariate linear regression analysis			Mendelian randomization analysis		
				β	SE	F	R^2	*p*	β	SE	*p*	β_{IV}	SE	*p*
BMI	serum ALT level[c]	1 SNP (rs2030323)	Model 1	0.307	0.070	19.2	0.005	<.001	0.088	0.003	<.001	-0.004	0.041	0.916
			Model 2	0.330	0.065	198	0.136	<.001	0.067	0.003	<.001	0.017	0.032	0.594
			Model 3	0.313	0.066	88.3	0.144	<.001	0.068	0.003	<.001	0.017	0.035	0.634
		GRS1[a]	Model 1	0.502	0.037	182	0.046	<.001	0.088	0.003	<.001	0.047	0.012	<.001
			Model 2	0.506	0.035	270	0.178	<.001	0.067	0.003	<.001	0.051	0.011	<.001
			Model 3	0.487	0.035	116	0.184	<.001	0.068	0.003	<.001	0.050	0.011	<.001
		GRS2[b] (*FTO/MC4R*)	Model 1	0.295	0.045	43.2	0.011	<.001	0.088	0.003	<.001	0.060	0.024	0.013
			Model 2	0.275	0.042	202	0.141	<.001	0.067	0.003	<.001	0.046	0.024	0.052
			Model 3	0.264	0.042	89.3	0.149	<.001	0.068	0.003	<.001	0.039	0.025	0.128

[a] GRS1: Using de novo 13 SNPs obtained from GWAS [b] GRS2: Using 13 candidate SNPs from the target genes (*FTO* and *MC4R*)
[c] Log-transformed [§] ALT, Alanine transaminase (continuous outcome)

Model 1: Crude model
Model 2: Adjusted for age and sex
Model 3: Adjusted for age, sex, smoking status, alcohol drinking, and exercise status

5. Sensitivity analyses

GRS generated from the candidate SNPs (GRS2) was a much weaker IV than GRS1. Hence, it was difficult to confidently confirm the same conclusion with the main results from GRS1. The Mendelian randomization analysis using GRS2 for BMI, however, assured the significant results of causal relationship between BMI and serum ALT level. All estimates from this type of GRS were also reported in Tables 8-11, along with the main results. When the same analyses excluding the heavy drinkers (N=3,268) and using the drinking amount information instead of drinking status were performed, the conclusion remained unchanged throughout the entire results (Tables 12-15).

Appendix B2 describes potential effects of genetic IVs (G) for serum adiponectin level or BMI on liver enzyme levels (Y), according to their effect sizes for each relevant intermediate phenotype. There was almost no change in effects (β coefficients) on the liver enzyme levels, regardless of the changes in effect sizes of genetic variants for adiponectin levels. However, there was a small alteration in effects on the final outcome, as SNPs showed different effect sizes on BMI.

A total of 36 and 37 SNPs that were significantly associated with elevated serum aminotransferase levels or serum ALT level (Y) in GWAS, respectively, were selected to conduct reciprocal analyses (Appendix A1 and A2). When the effects of these SNPs on exposure variables (X) were plotted, they revealed almost no effect on adiponectin levels

(Appendix B3). Contrastively, these SNPs seemed to have an influence on BMI with a proportional nature to the effect sizes for outcome variables (Y). Calculation of LD r^2 for every combination of SNPs on the same chromosome, across the groups of those determining adiponectin level, BMI, and liver enzyme levels, proved no evidence of any LD relationship.

Table 12. Association between adiponectin and abnormal liver enzyme level[§], among subjects without hepatitis B or C viral infection after exclusion of heavy drinkers

Explanatory variables	Outcome variable	Instrumental variable		Change in log-transformed adiponectin per increase of one risk allele or one unit of GRS					Observational multivariate logistic regression analysis			Mendelian randomization analysis	
				β	SE	F	R^2	*p*	OR	CI	*p*	OR	CI
Adiponectin[c]	elevated serum AST or ALT	1 SNP (rs4783244)	Model 1	-0.182	0.015	150	0.045	<.001	0.38	(0.31-0.45)	<.001	0.99	(0.42-2.35)
			Model 2	-0.180	0.014	240	0.184	<.001	0.50	(0.41-0.61)	<.001	1.02	(0.42-2.48)
			Model 3	-0.179	0.014	99.7	0.184	<.001	0.49	(0.40-0.61)	<.001	1.02	(0.41-2.53)
		GRS1[a]	Model 1	-0.032	0.003	141	0.043	<.001	0.38	(0.31-0.45)	<.001	0.76	(0.32-1.82)
			Model 2	-0.033	0.003	237	0.186	<.001	0.50	(0.41-0.61)	<.001	0.69	(0.29-1.63)
			Model 3	-0.034	0.003	99.7	0.187	<.001	0.49	(0.40-0.61)	<.001	0.68	(0.28-1.61)
		GRS2[b] (*ADIPOQ*)	Model 1	-0.059	0.008	56.3	0.017	<.001	0.38	(0.31-0.45)	<.001	1.06	(0.27-4.23)
			Model 2	-0.057	0.007	196	0.158	<.001	0.50	(0.41-0.61)	<.001	1.20	(0.27-5.27)
			Model 3	-0.056	0.007	82.1	0.158	<.001	0.49	(0.40-0.61)	<.001	1.14	(0.25-5.20)

[a] GRS1: Using de novo 7 SNPs obtained from GWAS [b] GRS2: Using 6 candidate SNPs from the target gene (*ADIPOQ*)

[c] Log-transformed

[§] Binary outcome defined as serum aminotransferase (AST; Aspartate aminotransferase, or ALT; Alanine transaminase) level ≥ 40IU/L

Model 1: Crude model

Model 2: Adjusted for age and sex

Model 3: Adjusted for age, sex, smoking status, amount of alcohol drinking, and exercise status

Table 13. Association between adiponectin and serum ALT[§] level, among subjects without hepatitis B or C viral infection after exclusion of heavy drinkers

Explanatory variables	Outcome variable	Instrumental variable		Change in log-transformed adiponectin per increase of one risk allele or one unit of GRS					Observational multivariate linear regression analysis			Mendelian randomization analysis		
				β	SE	F	R^2	*p*	β	SE	*p*	β_{IV}	SE	*p*
Adiponectin[c]	serum ALT level[c]	1 SNP (rs4783244)	Model 1	-0.182	0.015	150	0.045	<.001	-0.281	0.016	<.001	-0.031	0.078	0.693
			Model 2	-0.180	0.014	240	0.184	<.001	-0.132	0.016	<.001	-0.007	0.071	0.919
			Model 3	-0.179	0.014	99.7	0.184	<.001	-0.131	0.016	<.001	-0.003	0.073	0.964
		GRS1[a]	Model 1	-0.032	0.003	141	0.043	<.001	-0.281	0.016	<.001	-0.020	0.082	0.809
			Model 2	-0.033	0.003	237	0.186	<.001	-0.132	0.016	<.001	-0.051	0.070	0.465
			Model 3	-0.034	0.003	99.7	0.187	<.001	-0.131	0.016	<.001	-0.053	0.071	0.453
		GRS2[b] (*ADIPOQ*)	Model 1	-0.059	0.008	56.3	0.017	<.001	-0.281	0.016	<.001	-0.007	0.135	0.959
			Model 2	-0.057	0.007	196	0.158	<.001	-0.132	0.016	<.001	0.035	0.123	0.777
			Model 3	-0.056	0.007	82.1	0.158	<.001	-0.131	0.016	<.001	0.015	0.127	0.903

[a] GRS1: Using de novo 7 SNPs obtained from GWAS [b] GRS2: Using 6 candidate SNPs from the target gene (*ADIPOQ*)
[c] Log-transformed [§] ALT, Alanine transaminase (continuous outcome)

Model 1: Crude model
Model 2: Adjusted for age and sex
Model 3: Adjusted for age, sex, smoking status, amount of alcohol drinking, and exercise status

Table 14. Association between body mass index and abnormal liver enzyme level[§], among subjects without hepatitis B or C viral infection after exclusion of heavy drinkers

Explanatory variables	Outcome variable	Instrumental variable		Change in body mass index per increase of one risk allele or one unit of GRS					Observational multivariate logistic regression analysis			Mendelian randomization analysis	
				β	SE	F	R^2	*p*	OR	CI	*p*	OR	CI
BMI	elevated serum AST or ALT	1 SNP (rs2030323)	Model 1	0.297	0.076	15.3	0.004	<.001	1.42	(1.36-1.47)	<.001	1.09	(0.92-1.29)
			Model 2	0.313	0.071	167	0.133	<.001	1.40	(1.34-1.46)	<.001	1.15	(0.87-1.51)
			Model 3	0.290	0.072	75.2	0.142	<.001	1.41	(1.35-1.47)	<.001	1.10	(0.65-1.87)
		GRS1[a]	Model 1	0.512	0.040	162	0.048	<.001	1.42	(1.36-1.47)	<.001	1.29	(1.10-1.52)
			Model 2	0.514	0.038	230	0.177	<.001	1.40	(1.34-1.46)	<.001	1.32	(1.12-1.57)
			Model 3	0.499	0.038	101	0.184	<.001	1.41	(1.35-1.47)	<.001	1.32	(1.11-1.57)
		GRS2[b] (*FTO/MC4R*)	Model 1	0.311	0.048	42.6	0.013	<.001	1.42	(1.36-1.47)	<.001	1.24	(0.81-1.89)
			Model 2	0.289	0.044	173	0.141	<.001	1.40	(1.34-1.46)	<.001	1.20	(0.85-1.68)
			Model 3	0.272	0.045	77.7	0.149	<.001	1.41	(1.35-1.47)	<.001	1.21	(0.84-1.74)

[a] GRS1: Using de novo 13 SNPs obtained from GWAS [b] GRS2: Using 13 candidate SNPs from the target genes (*FTO* and *MC4R*)

[§] Binary outcome defined as serum aminotransferase (AST; Aspartate aminotransferase, or ALT; Alanine transaminase) ≥ 40IU/L

Model 1: Crude model

Model 2: Adjusted for age and sex

Model 3: Adjusted for age, sex, smoking status, amount of alcohol drinking, and exercise status

Table 15. Association between body mass index and serum ALT[§] level, among subjects without hepatitis B or C viral infection after exclusion of heavy drinkers

Explanatory variables	Outcome variable	Instrumental variable		Change in body mass index per increase of one risk allele or one unit of GRS					Observational multivariate linear regression analysis			Mendelian randomization analysis		
				β	SE	F	R^2	*p*	β	SE	*p*	β_{IV}	SE	*p*
BMI	serum ALT level[c]	1 SNP (rs2030323)	Model 1	0.297	0.076	15.3	0.004	<.001	0.087	0.003	<.001	-0.012	0.047	0.795
			Model 2	0.313	0.071	167	0.133	<.001	0.068	0.003	<.001	0.006	0.038	0.866
			Model 3	0.290	0.072	75.2	0.142	<.001	0.069	0.003	<.001	-0.006	0.043	0.892
		GRS1[a]	Model 1	0.512	0.040	162	0.048	<.001	0.087	0.003	<.001	0.041	0.013	0.002
			Model 2	0.514	0.038	230	0.177	<.001	0.068	0.003	<.001	0.044	0.012	<.001
			Model 3	0.499	0.038	101	0.184	<.001	0.069	0.003	<.001	0.041	0.012	0.001
		GRS2[b] (*FTO/MC4R*)	Model 1	0.311	0.048	42.6	0.013	<.001	0.087	0.003	<.001	0.065	0.024	0.007
			Model 2	0.289	0.044	173	0.141	<.001	0.068	0.003	<.001	0.050	0.024	0.035
			Model 3	0.272	0.045	77.7	0.149	<.001	0.069	0.003	<.001	0.045	0.026	0.082

[a] GRS1: Using de novo 13 SNPs obtained from GWAS [b] GRS2: Using 13 candidate SNPs from the target genes (*FTO* and *MC4R*)
[c] Log-transformed [§] ALT, Alanine transaminase (continuous outcome)

Model 1: Crude model
Model 2: Adjusted for age and sex
Model 3: Adjusted for age, sex, smoking status, amount of alcohol drinking, and exercise status

III. Discussion

1. Discussion of Study Results

In this population-based study, we provided evidence of a causal effect of obesity on elevation in serum aminotransferase level, which suggests hepatocyte injury. However, the results did not provide any consistent evidence for serum adiponectin as a causal factor for hepatocyte protection or reducing serum liver enzyme level.

A. Review of previous studies and biological plausibility: Adiponectin

Over the past decade, studies on the association among obesity, insulin sensitivity, and obesity-related metabolic disorders including fatty liver disease or NASH have been actively conducted, accompanied by suggestions of putative mechanisms; but most aspects are still unclear. One of the major controversies is the role and mechanism of adipokines such as adiponectin in the target organ. Some studies have implied these hormones may act as insulin sensitizers, which can reduce insulin resistance. Many researchers have even suggested the hormones as potential therapeutic or pharmacologic targets against diabetes or related disorders. Along with this claim, it has been argued that adiponectin protects liver cells from fat accumulation and fibrosis. This means it has an anti-steatotic, anti-inflammatory, and anti-fibrogenic effect and may lower the risk of fatty liver disease, as well as the risk of hepatocellular carcinoma.

Results from observational studies and animal experiments have supported this claim.[17,43,44] Nonetheless, previous studies were not free from confounders or reverse causation. Instead of using an experimental group that only differed from the control group in circulating adiponectin levels, genetically or pharmacologically manipulated obese and diabetogenic mice served as the experimental group.[45] Therefore, to address these problems, some Mendelian randomization studies have been proposed to investigate the causal relationship between adiponectin and diabetes or related disease conditions.

The most recent Mendelian randomization study, which examined the association of blood adiponectin levels with insulin resistance and type 2 diabetes, was conducted on about 30,000 (up to 31,000) subjects from 13 European studies in 2013.[45] It was a large-scale study that included the study group of 942 subjects,[46] from which other researchers concluded that there is a causal relationship on the same topic, just before this study. In this larger study, researchers also used the genotype of the *ADIPOQ* gene and GRS; they argued that the causal association between adiponectin and insulin resistance or type 2 diabetes could not be confirmed, because the IV analysis revealed that genetically determined low levels of adiponectin was not associated with increased insulin resistance and type 2 diabetes risk. This study added to the results of the animal study that showed genetically reduced adiponectin does not cause increased insulin resistance, and

suggested even a bidirectional relationship between insulin resistance and hypoadiponectinemia in humans.[47]

The authors of these studies explained it as follows: insulin resistance causes hyperinsulinemia, which in turn reduces serum adiponectin levels. Although such an explanation is inconsistent with the current view of adiponectin as an insulin-sensitizing hormone, it is supported by research that demonstrated decreased adiponectin level in participants under hyperinsulinemic conditions or receiving high-dose insulin infusion; other studies reported markedly elevated adiponectin level, (1) in people with functional loss of insulin receptors, which can be perceived as a constant hypoinsulinemic condition even though it accompanies extremely high insulin resistance, or (2) in patients with type 1 diabetes who actually exhibit low insulin levels.[45,48-56]

For the liver (when we extend the concept of insulin resistance to the liver), adiponectin has been regarded as having an inverse correlation with hepatic fat and hepatic insulin resistance in diabetic patients; in healthy people, low adiponectin concentration has been significantly associated with increased serum ALT and GGT levels, suggesting the role of adiponectin in hepatocyte injury or protection pathway.[57-60] However, by focusing on the causal relationship, our study demonstrated that it was just an association rather than a prior contribution of adiponectin onto liver enzyme levels or hepatocyte injury. This conclusion also corresponds with the interpretations of previous study results, which

disagreed with a causal relationship between adiponectin level and systematic insulin resistance, along with several biological mechanisms.

B. Review of previous studies and biological plausibility: Obesity (BMI)

Likewise, there has been almost no Mendelian randomization study that directly investigated liver function or liver diseases as outcomes, with obesity or other similar conditions as a potential causal factor. However, the relationships between obesity as an exposure (potential cause of disease) and other abnormalities in cardiometabolic traits or associated diseases like fasting insulin level, triglyceride or HDL-cholesterol level, blood pressure, metabolic syndrome, type 2 diabetes, ischemic heart disease, or stroke as a respective outcome, have been tested through previous Mendelian randomization studies estimating whether they are causal associations or not.[61-66] A relatively recent large-scale Mendelian randomization analysis regarding the association between elevated BMI and various cardiometabolic phenotypes has not only confirmed these causal relationships between adiposity and diverse cardiovascular diseases through the SNPs of the *FTO* gene, but has also provided new evidence for adiposity as a causal risk factor in relation with increased ALT and GGT.[62]

Results of the present study provides additional evidence for a causal relationship between obesity, assessed as BMI, and potential hepatocyte injury or liver disease, assessed by ALT and AST. Indeed, obesity is now recognized as a major independent risk

factor for NAFLD.[9] Although the initial point of alteration still remains controversial, insulin resistance in adipose, or peripheral tissue is considered to be the most critical and important factor in the pathogenesis of NAFLD.[2,4,67] With regard to obesity, increased free fatty acids originating from excess adipose tissue inhibit the activation or expression of insulin receptors and glucose transporter proteins on the cell surface; along with inflammatory cytokine action produced by adipose tissues, insulin sensitivity decreases and insulin resistance may occur.[68]

This insulin resistance, induced from abnormally enlarged inflammatory adipose tissue, causes significant changes in lipid metabolism such as increased peripheral lipolysis, increased triglyceride synthesis, and increased fatty acid uptake in hepatocytes.[2,69,70] The hepatic synthesis of triglycerides from fatty acids introduced from the blood, which is a basic function of liver cells that can be promoted by insulin,[71,72] may be accelerated in patients with increased insulin resistance and subsequent hyperinsulinemia.

Both of the above actions can cause triglyceride accumulation in the liver, which in turn leads to a further shift in liver cell metabolism that induces change from the carbohydrates absorbed through the blood to the synthesis of fatty acids (de novo lipogenesis), and subsequently to the beta-oxidation of free fatty acids.[71,72] Such a shift in hepatocyte metabolism, which predisposes them to generate more fatty acids from glucose and its derivatives, is also a process that can be promoted by insulin; it has been

reported that this shift toward fatty acid synthesis and oxidation is actually observed in patients with increased insulin resistance, supporting the suggested mechanism.[71-74]

All of the above explanations that link obesity to increased lipid accumulation and oxidation in the liver mediated by insulin resistance would be an important axis that explains much of the liver cell injury caused by obesity. To think about other causes that lead to elevated liver enzyme levels, we can completely rule out the possibility of hepatitis B or C viral infection from the study design step; we can also disregard the potential effects of alcohol to liver enzymes because it was adjusted for by statistical modeling or restricted from the start in sensitivity analyses. Hence, except for relatively rare or miscellaneous factors that lead to mild elevation in aminotransferase levels such as hemochromatosis, gallstones, and cholecystitis, hepatic steatosis and NASH are the potential causes for slightly abnormal liver enzyme levels.[8,75]

Insulin resistance is considered to play an important role in the occurrence of NASH as well,[76-78] but is not a feature of all NASH patients. Indeed, it has been documented that NASH is easily observed in patients who are not obese or have normal glucose tolerance, and it is suggested that NASH could be a more heterogeneous disease that originating from one or more risk factors.[2,76] In other words, NASH can develop through other mechanisms besides insulin resistance that occurs with hepatic lipid accumulation, in

contrast to simple steatosis that almost entirely develops from alterations in insulin sensitivity.

2. Discussion of Study Methods

By applying the Mendelian randomization approach, we were able to test for a causal relationship between adiposity, adiponectin, and elevation of liver enzymes, with the methodological strength in avoiding bias from potential confounders and reverse causation. Analyses were conducted within a relatively large study sample, which consisted of a randomly selected control group from the population-based cohort using the novel microarray chip reflecting ethnic specificity in genomic data for Korean people.

However, there are also several limitations regarding the Mendelian randomization method.[39] First, confounding of genotype-intermediate phenotype (i.e. exposure of interest) – disease associations can occur. One of the mechanisms for this is LD, which refers to the association between different SNPs located in different loci. According to Smith and Ebrahim, the following two situations can bring about potential confounding in the study: if the SNPs determining the intermediate phenotype, or any other polymorphisms that are in LD with these SNPs, (1) affect the disease outcome directly through another pathway (by affecting through different ways to outcome, or affecting on common metabolic pathway that is related to both of the intermediate phenotype and the final outcome); or (2) affect the behavioral factors that may influence the final outcome

(through LD relationships between the SNPs affecting the intermediate phenotype and the SNPs affecting problematic behaviors, or through pleiotropic effects of those SNPs). These confounding caused by LD is closely linked with the third assumption of Mendelian randomization analysis (presented in the methods section), also known as the 'exclusion restriction'. However, it is known that this assumption cannot be fully verified empirically.[79,80]

To assess the problem of genetic LD in this study, we tested all LD values that can occur among the variants of two intermediate phenotypes (i.e. adiponectin level and BMI) and the final outcome (Figure 6). Since it was possible to list the SNPs that were significantly associated with these intermediate phenotypes and outcome variables (G1, G2 and G3), any LD among them could be directly observed through an appropriate analysis. All three combinations of SNPs located in the same chromosome demonstrated r^2 of LD less than 0.002 and were able to prove their mutual independences.

Another possible confounding factor in the genotype–intermediate phenotype–disease relationship, other than LD, is population stratification. This confounding is related to a violation of the second assumption for the Mendelian randomization study. However, the study population used in this analysis was a relatively homogenous group, consisting of mono-ethnic healthy people who were confirmed to be negative for the HBV and HCV

virus infection; hence it was unlikely that different distributions in genotype, intermediate phenotype, and disease existed between subgroups of this study population.

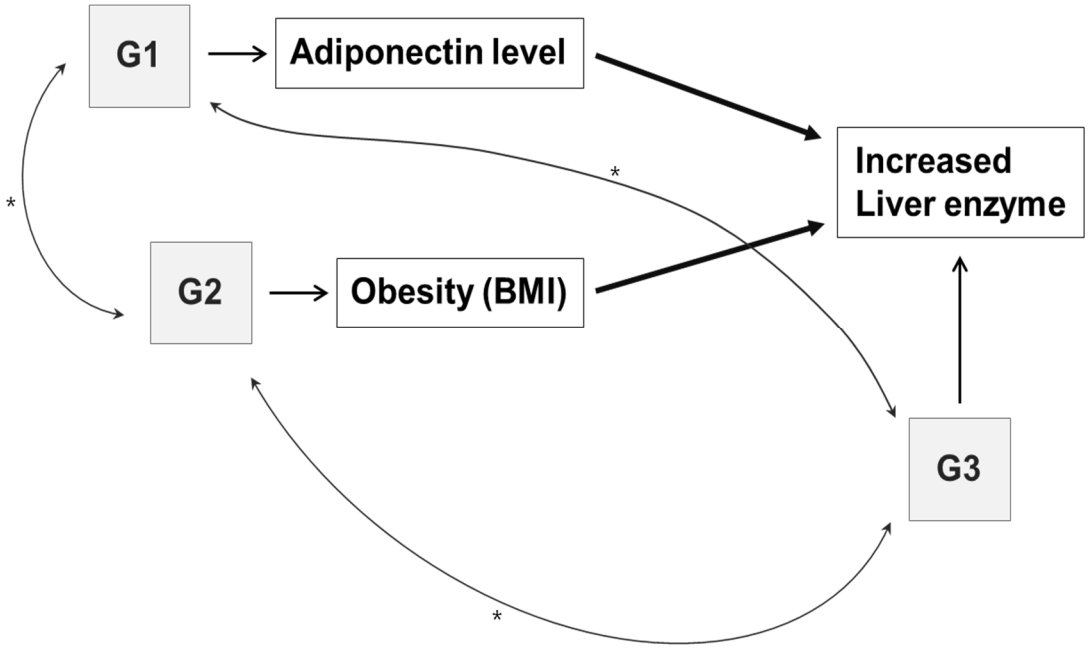

Figure 6. Schematic expression of LD assessment (bidirectional arrows*) for genetic variants affecting two intermediate phenotypes and the outcome of interest

Second, pleiotropy and multi-function of genes can also exist. In the current study, the polymorphisms that influence adiponectin or BMI may also influence other phenotypes or a variety of pathophysiological processes, and involvement of such SNPs in the study would lead to some confusion in interpreting the final results by altering the effect size; it is difficult to interpret the association between genetic polymorphism and phenotypes under such a situation.

These problems of confounding and pleiotropy could be assessed by measuring potential confounders in association with genetic data[81] as shown in Tables 6 and 7. Besides, a graphical approach described in Appendix B2 could suggest lower possibilities for the presence of other routes between the genetic IVs and the final outcome, particularly for the IVs of adiponectin level. In addition, results of reciprocal analyses (Appendix B3) implied that some of SNPs that determine liver enzyme levels would have an influence on BMI as well. They can be accepted as potential genetic confounders, and bidirectional causal relationship may exist. Further study and analysis would be worth a try with an investigation for more clear relationships regarding those complex mechanisms (Figure 7).

Third, canalization or developmental compensation might occur when an organism is influenced by a polymorphic genotype and altering the developmental process, such as during fetal development period to buffer against genetic effect. This problem is more difficult to study than other limitations mentioned above. However, most examples of

canalization are related to striking genetic or environmental insults, suggesting that single polymorphisms inducing just a small variation in phenotypes may not be sufficient to make compensatory changes.[81]

Fourth, the time-varying nature of the exposure variables, the possibility of gene-environmental interaction, a measurement error in both exposure and outcome variables, and other potential biases could influence the study results. These biases have a greater possibility of influencing the results toward a false-positive, rather than a negative result [80]; it is probably due to the majority of these biases could have differential nature of misclassification.

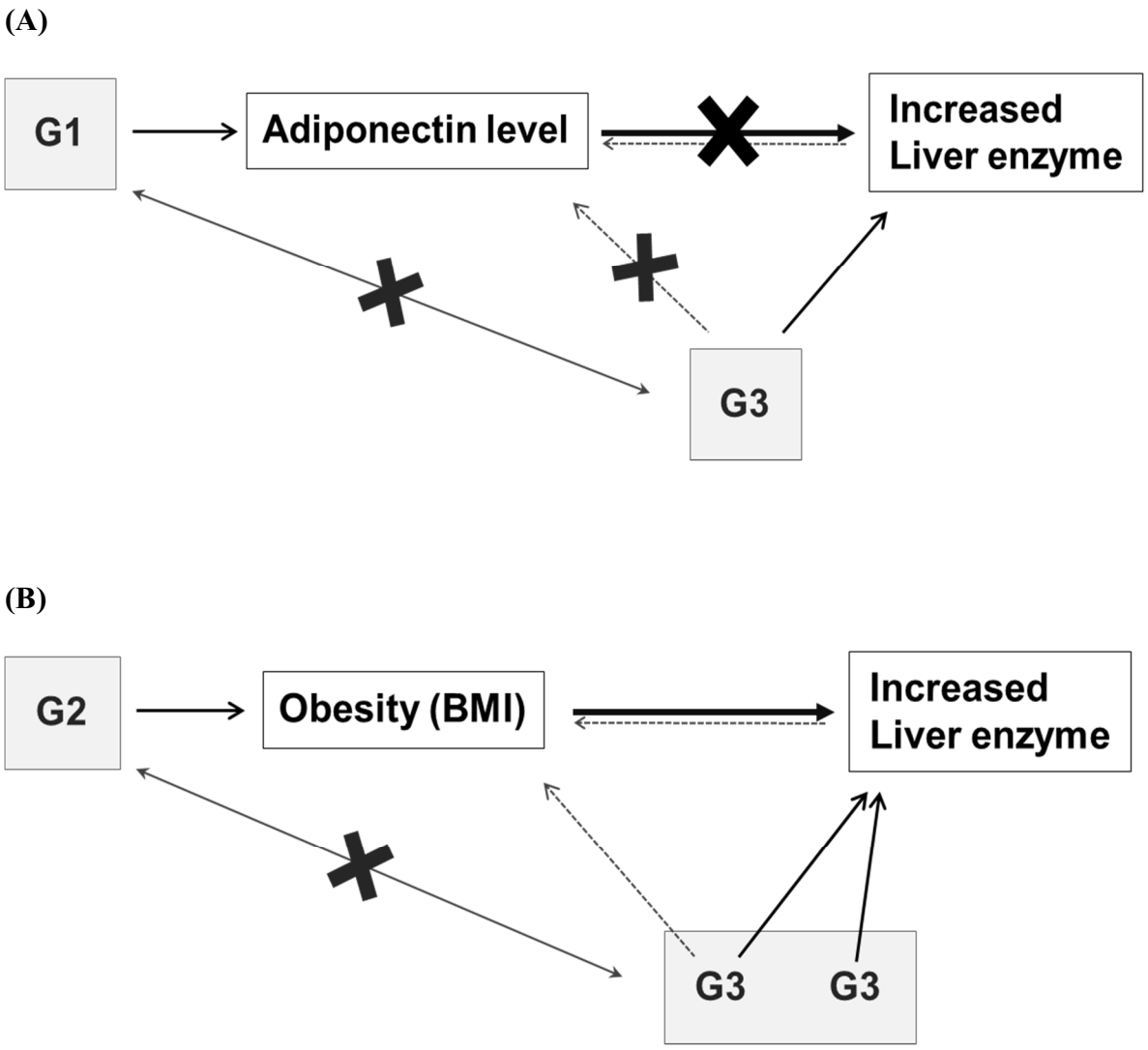

Figure 7. Causal diagram and the result of Mendelian randomization analysis on (A) serum adiponectin level and (B) body mass index for liver enzyme levels, with the findings of reciprocal approach, and LD assessment among the variants

3. Comprehensive Discussion

There are some discussion points through the entire process of the study.

First, the lists of significant SNPs for adiponectin and BMI obtained from this study were quite different from those in previous studies. As for the GWAS results regarding adiponectin, only two SNPs of the *ADIPOQ* gene, which is the major adiponectin-related gene that has been reported mainly in Western populations, were included among the top 20 SNPs; moreover, their effects were found to be weak. Most of the stronger effects were shown with the SNPs located on the *CDH13* gene that has been mainly reported in Asian populations.[82-85] Similarly, in the GWAS results regarding BMI, there were no significant SNPs from familiar obesity-related genes such as *FTO* or *MC4R* found on the list; however, a few SNPs on the *BDNF* gene, which has been reported in a relatively small number of studies,[86-89] were included in the top 20 SNPs list.

The following explanations would be applicable to these results: (1) they are ethnic-specific results that obtained from the Asian (in particular, Korean) population, and (2) they are normal control group-specific outcomes derived from the sub-population consisting of participants who were confirmed as HBV and HCV-negatives, particularly within the Korean population in which the prevalence of chronic hepatitis virus infection is typically high. For the first sensitivity analysis regarding this problem, we obtained results from the Mendelian randomization analysis by using the separate sets of SNPs

selected from *ADIPOQ*,[90,91] *FTO* and *MC4R* [92,93] gene regions, respectively, and compared them to the original results. For verification of the second hypothesis, we obtained GWAS results from the entire subcohort population prior to the selection of subjects who had negative result for hepatitis virus test. When comparing them with the original results, there was a clear difference in the BMI-related SNPs whereas adiponectin-related SNPs did not show significant differences. This BMI-related SNP list obtained from the entire subcohort included several polymorphisms located on the *MC4R* gene.

Second, the effect of genotypes that determine the intermediate phenotype traits in the present study, especially for the BMI variable, seemed to polygenic, and this genetic heterogeneity could be a relative weakness in the genotype-exposure association.[41] However, the significance or explanatory power of the genetic variables, estimated by the F statistic, was enough to perform the analysis, and it was well maintained even for the GRS, which was generated using multiple genetic variants.

Third, the low statistical power for the Mendelian randomization method was an inevitable limitation,[81] which can be considered as a major cause of negative results in the analyses performed using single SNPs. However, by using multiple polymorphisms via combined single values of GRS, we were able to pull up the power of study and get positive results for estimation in regard to BMI.

Fourth, as VanderWeele et al. suggested, Mendelian randomization design has very strong assumptions and asymmetric probability distribution between false negatives and false positives.[80] This may result in negative results being more robust and valuable than positive results, granted that negative results would require a sufficiently large sample size in order to be reliable. When these conditions and assumptions are satisfied, the negative result for adiponectin in this study could be more meaningful.

Fifth, the methodologies for causal inference by controlling confounders based on the counterfactual model have a theoretical ability to control both the known and unknown confounders. However, in the field of epidemiology, it should be noted that this is just one of the approaches for causal inference. It is impossible to determine causation by any single method or study.[94] The results of this study need to be understood based upon the strengths and limitations of the method used, as well as evaluated in the light of 'pragmatic pluralism' and whether this approach was suitable for ‘inference to the best explanation’, according to the emphasis of experts.[94,95]

IV. Conclusion

The present study investigated causal association using a population-based cohort composed of participants who had no prevalent disease and confirmed as negative to hepatitis B and C virus infection. Through Mendelian randomization analysis, we were able to provide evidence of a causal effect of obesity on elevation in serum aminotransferase levels, which suggests hepatocyte injury. However, the results did not provide any consistent evidence for serum adiponectin as a causal factor for hepatocyte protection or reducing serum liver enzyme level. The study results could not disprove the effectiveness of adiponectin or its suggested mechanism on metabolic traits. However, our findings help understand possible pathogenetic mechanisms that start from excessive adiposity and are linked to liver injury: obesity seems to be a causal factor, while decreased adiponectin seems to be an intermediate result rather than a cause.

The present study was the first Mendelian randomization study regarding liver damage assessed by aminotransferases levels in relation to obesity and adiponectin using genetic variants specific for the Asian population. According to our results, we suggest adiposity as the primary target for both preventive and therapeutic approaches regarding obesity-related liver damage or metabolic liver diseases.

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Appendix

Appendix A. Supplementary Tables

A1. A list of 36 single nucleotide polymorphisms strongly relevant to the elevation of AST or ALT level (binary outcome)

Chr	SNP	Risk allele	Other allele	RAF	per allele odds ratio for elevated LFT (≥ 40 IU/L)		per allele change in log-transformed adiponectin		per allele change in body mass index	
					OR	95% CI	beta	SE	beta	SE
18	rs148732782	A	G	0.023	2.25	(1.60-3.16)	-0.0383	(0.0425)	0.3704	(0.2265)
2	rs139657612	A	G	0.013	2.84	(1.83-4.40)	-0.0727	(0.0570)	0.8276	(0.3040)
17	rs9302960	G	C	0.278	1.38	(1.20-1.59)	-0.0042	(0.0147)	0.1446	(0.0779)
3	rs709157	A	G	0.021	2.14	(1.49-3.07)	-0.0210	(0.0451)	0.2036	(0.2385)
14	rs2877753	G	A	0.394	1.39	(1.22-1.59)	-0.0318	(0.0133)	0.1177	(0.0702)
3	rs9637430	C	T	0.561	1.34	(1.17-1.54)	-0.0145	(0.0130)	0.1039	(0.0692)
16	rs1558679	C	T	0.076	1.59	(1.27-1.99)	-0.0111	(0.0253)	0.0570	(0.1338)
17	rs4795294	A	C	0.372	1.32	(1.15-1.50)	0.0122	(0.0135)	0.1117	(0.0717)
21	rs2836467	T	C	0.028	1.99	(1.42-2.79)	0.0172	(0.0408)	0.1637	(0.2144)
10	rs138544218	T	C	0.026	2.01	(1.43-2.84)	0.0474	(0.0411)	-0.0275	(0.2193)
11	rs74493704	A	C	0.012	2.58	(1.62-4.10)	-0.0411	(0.0603)	0.1911	(0.3169)
6	rs9474312	T	C	0.765	1.40	(1.19-1.66)	-0.0002	(0.0152)	0.1620	(0.0805)
5	rs74436539	A	G	0.017	2.34	(1.56-3.52)	0.0227	(0.0513)	0.4181	(0.2725)
16	rs147990649	T	C	0.020	2.17	(1.48-3.19)	0.0515	(0.0481)	0.4875	(0.2505)
22	rs2281135	A	G	0.412	1.33	(1.16-1.52)	0.0040	(0.0132)	0.0406	(0.0699)
2	rs60825457	A	G	0.259	1.33	(1.15-1.54)	-0.0403	(0.0150)	0.1508	(0.0793)
13	rs9556106	G	A	0.535	1.32	(1.15-1.51)	-0.0071	(0.0132)	0.0324	(0.0698)
22	rs5748561	T	G	0.217	1.39	(1.19-1.62)	0.0089	(0.0159)	0.1883	(0.0841)
4	rs118080016	A	C	0.028	2.07	(1.49-2.88)	-0.0630	(0.0401)	0.0542	(0.2124)
8	rs77283999	A	G	0.018	2.16	(1.45-3.23)	0.0260	(0.0502)	0.4158	(0.2641)
21	rs75497364	T	C	0.046	1.71	(1.31-2.25)	0.0179	(0.0316)	-0.0113	(0.1669)
2	rs13028301	C	T	0.602	1.31	(1.14-1.51)	0.0021	(0.0133)	0.0837	(0.0706)
2	rs13003781	A	G	0.583	1.31	(1.14-1.50)	-0.0031	(0.0131)	0.1003	(0.0697)
18	rs9952685	A	G	0.160	1.41	(1.19-1.65)	-0.0171	(0.0174)	0.1632	(0.0923)

3	rs3773392	T	C	0.589	1.33	(1.16-1.53)	-0.0219	(0.0131)	0.0888	(0.0696)
20	rs6128136	A	G	0.574	1.34	(1.16-1.54)	-0.0120	(0.0135)	0.1602	(0.0712)
4	rs118007697	T	C	0.021	2.19	(1.51-3.18)	-0.0158	(0.0460)	0.4063	(0.2443)
8	rs7844770	A	G	0.245	1.29	(1.11-1.50)	-0.0011	(0.0153)	0.0452	(0.0809)
22	rs3761472	G	A	0.404	1.31	(1.15-1.50)	0.0006	(0.0133)	0.0304	(0.0706)
1	rs10873661	A	G	0.663	1.31	(1.13-1.51)	-0.0034	(0.0139)	0.0154	(0.0735)
1	rs3790370	G	A	0.301	1.32	(1.15-1.51)	0.0117	(0.0142)	0.0875	(0.0753)
3	rs17015485	A	G	0.897	1.61	(1.25-2.08)	-0.0133	(0.0219)	0.2171	(0.1155)
1	rs79622060	A	C	0.046	1.49	(1.13-1.97)	-0.0082	(0.0311)	0.3480	(0.1652)
11	rs118170751	G	A	0.058	1.54	(1.21-1.97)	-0.0038	(0.0275)	0.1805	(0.1463)
1	rs6682050	C	T	0.155	1.36	(1.15-1.61)	0.0153	(0.0180)	-0.0627	(0.0956)
15	rs149192877	G	T	0.014	2.18	(1.40-3.41)	0.0071	(0.0554)	0.2103	(0.2934)

Adjusted for age, sex, and alcohol drinking status

AST, Aspartate aminotransferase; ALT, Alanine transaminase; RAF, Risk allele frequency

LFT, Liver function tests, i.e. serum aminotransferase (AST or ALT) levels

A2. A list of 37 single nucleotide polymorphisms strongly relevant to serum ALT level (continuous outcome)

Chr	SNP	Risk allele	Other allele	RAF	per allele change in log-transformed ALT		per allele change in log-transformed adiponectin		per allele change in body mass index	
					beta	SE	beta	SE	beta	SE
12	rs671	G	A	0.840	0.0546	(0.0169)	0.0035	(0.0180)	0.1067	(0.0953)
22	rs2281135	A	G	0.412	0.0598	(0.0124)	0.0040	(0.0132)	0.0406	(0.0699)
12	rs2074356	G	A	0.851	0.0544	(0.0175)	-0.0087	(0.0186)	0.1205	(0.0984)
22	rs3761472	G	A	0.404	0.0571	(0.0125)	0.0006	(0.0133)	0.0304	(0.0706)
22	rs12483959	A	G	0.410	0.0557	(0.0125)	0.0102	(0.0133)	0.0523	(0.0701)
22	rs2143571	A	G	0.409	0.0540	(0.0124)	0.0059	(0.0132)	0.0314	(0.0701)
22	rs2073080	T	C	0.412	0.0514	(0.0124)	0.0044	(0.0132)	0.0326	(0.0697)
12	rs12229654	T	G	0.857	0.0464	(0.0178)	0.0043	(0.0189)	0.0608	(0.1000)
12	rs12825782	C	T	0.947	0.0848	(0.0280)	-0.0158	(0.0297)	0.0842	(0.1576)
3	rs141040283	A	G	0.021	0.1473	(0.0434)	-0.0331	(0.0460)	0.8132	(0.2435)
10	rs72794433	A	G	0.853	0.0778	(0.0177)	-0.0159	(0.0187)	0.2418	(0.0994)
12	rs11066453	A	G	0.874	0.0591	(0.0189)	0.0135	(0.0200)	0.0267	(0.1062)
17	rs16976928	C	T	0.911	0.0840	(0.0217)	-0.0212	(0.0229)	0.3346	(0.1218)
3	rs13066322	T	C	0.366	0.0397	(0.0129)	-0.0189	(0.0137)	0.1538	(0.0723)
2	rs4853928	C	T	0.071	0.0981	(0.0242)	-0.0529	(0.0257)	0.3306	(0.1363)
18	rs2846607	G	A	0.747	0.0533	(0.0143)	-0.0088	(0.0152)	0.0987	(0.0804)
9	rs3124299	C	T	0.576	0.0510	(0.0126)	-0.0138	(0.0133)	0.1120	(0.0710)
14	rs148953101	T	C	0.017	0.1435	(0.0479)	0.0282	(0.0507)	0.3835	(0.2692)
11	rs78043680	A	G	0.018	0.1652	(0.0474)	0.0132	(0.0498)	0.5443	(0.2661)
12	rs2072134	G	A	0.882	0.0613	(0.0194)	0.0053	(0.0205)	0.0100	(0.1091)
2	rs60825457	A	G	0.259	0.0460	(0.0141)	-0.0403	(0.0150)	0.1508	(0.0793)
11	rs118043978	A	G	0.049	0.0973	(0.0288)	-0.0164	(0.0306)	0.0269	(0.1617)
6	rs9474312	T	C	0.765	0.0481	(0.0143)	-0.0002	(0.0152)	0.1620	(0.0805)
1	rs34110867	C	T	0.139	0.0488	(0.0178)	0.0067	(0.0188)	0.0839	(0.1000)
14	rs11159377	C	A	0.116	0.0761	(0.0194)	-0.0060	(0.0205)	0.1547	(0.1089)
22	rs6006611	G	A	0.523	0.0497	(0.0124)	0.0032	(0.0131)	0.0295	(0.0695)
10	rs7083899	A	G	0.091	0.0963	(0.0214)	-0.0303	(0.0228)	0.1360	(0.1204)
17	rs9302960	G	C	0.278	0.0469	(0.0139)	-0.0042	(0.0147)	0.1446	(0.0779)

14	rs17094134	T	C	0.905	0.0819	(0.0213)	-0.0502	(0.0225)	0.0904	(0.1200)
7	rs140414042	A	C	0.014	0.1742	(0.0532)	0.0120	(0.0564)	0.2838	(0.2987)
10	rs12360216	A	G	0.083	0.0801	(0.0222)	-0.0409	(0.0236)	0.2513	(0.1247)
4	rs76520795	C	T	0.943	0.1024	(0.0274)	-0.0288	(0.0290)	0.2333	(0.1543)
12	rs12580178	A	G	0.131	0.0760	(0.0183)	-0.0429	(0.0194)	0.2022	(0.1028)
2	rs997451	C	A	0.038	0.1042	(0.0317)	-0.0342	(0.0335)	0.1344	(0.1782)
5	rs34224742	G	A	0.974	0.1223	(0.0389)	-0.0395	(0.0413)	0.3842	(0.2188)
4	rs118007697	T	C	0.021	0.1699	(0.0434)	-0.0158	(0.0460)	0.4063	(0.2443)
6	rs960298	G	A	0.588	0.0383	(0.0124)	0.0102	(0.0132)	0.1320	(0.0699)

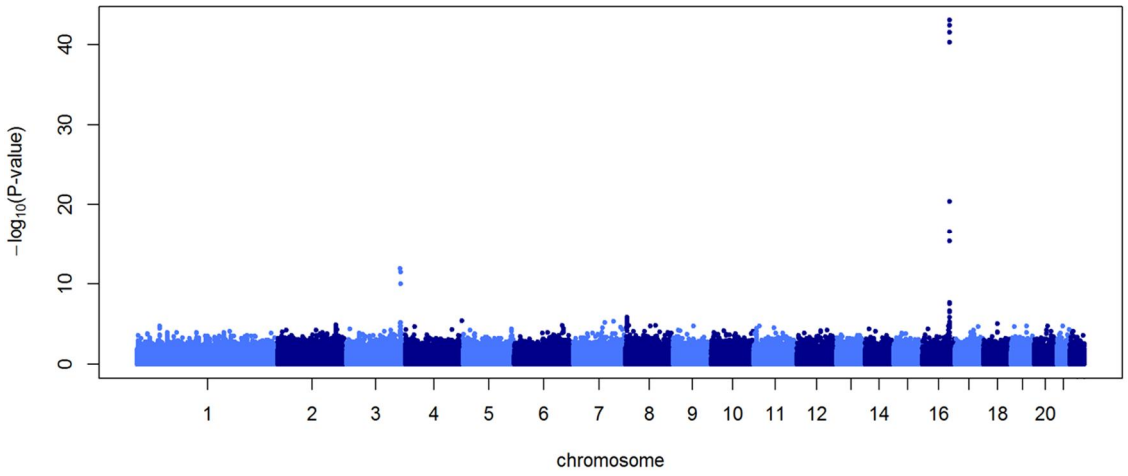
Adjusted for age, sex, and alcohol drinking status

ALT, Alanine transaminase; RAF, Risk allele frequency

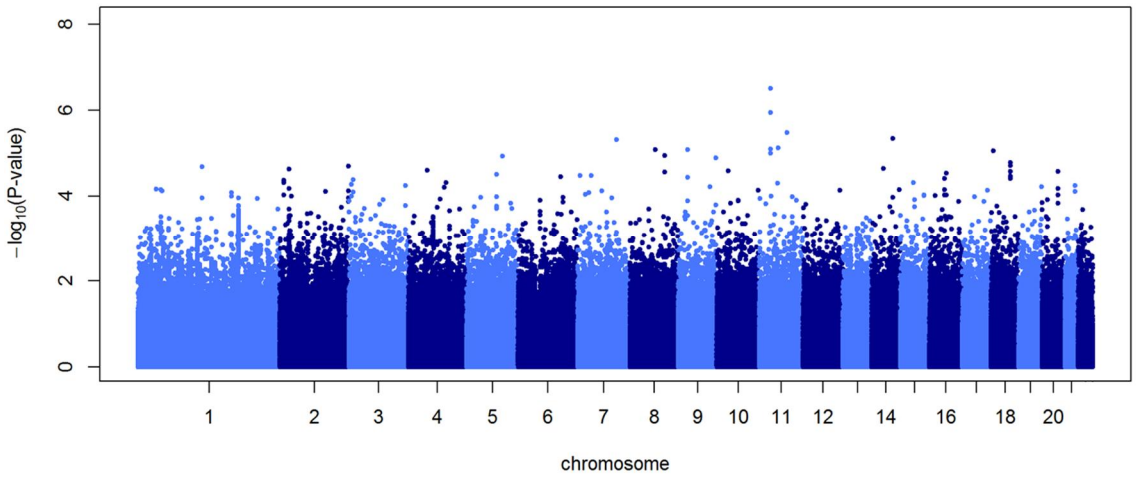
Appendix B. Supplementary Figures

B1. Manhattan plots of the *p*-values regarding (A) serum adiponectin level and (B) body mass index

(A)



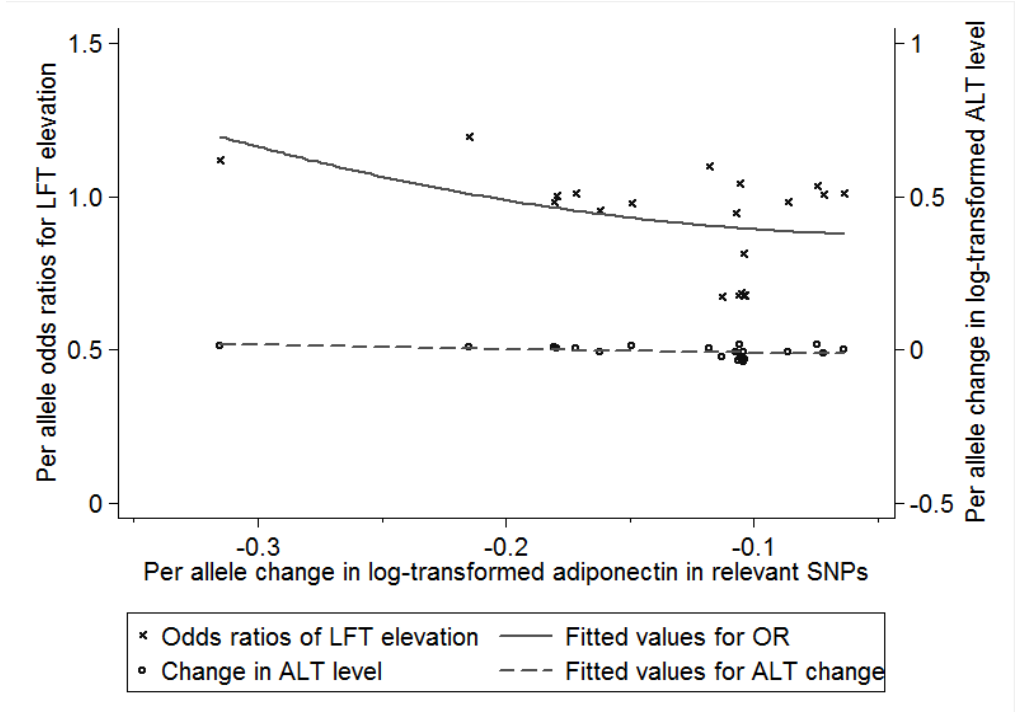
(B)



B2.

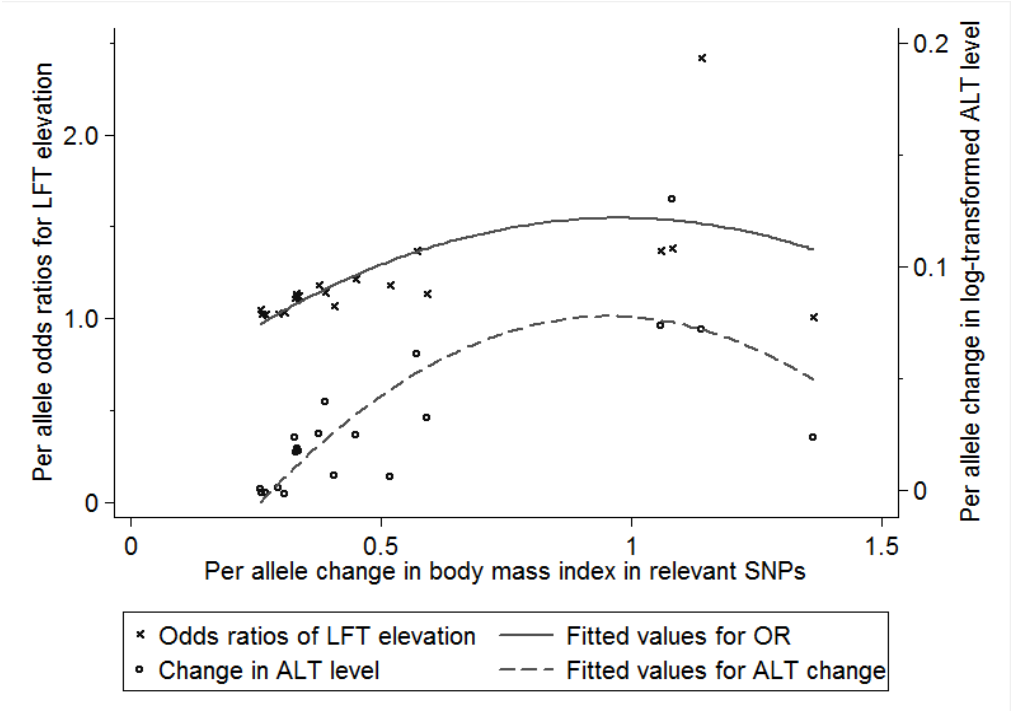
Effects (β coefficients) of (A) serum adiponectin and (B) body mass index determining SNPs on the risk of LFT elevation and serum ALT level

(A)



LFT, Liver function tests, i.e. serum aminotransferase (both of AST and ALT) levels; AST, Aspartate aminotransferase; ALT, Alanine transaminase

(B)

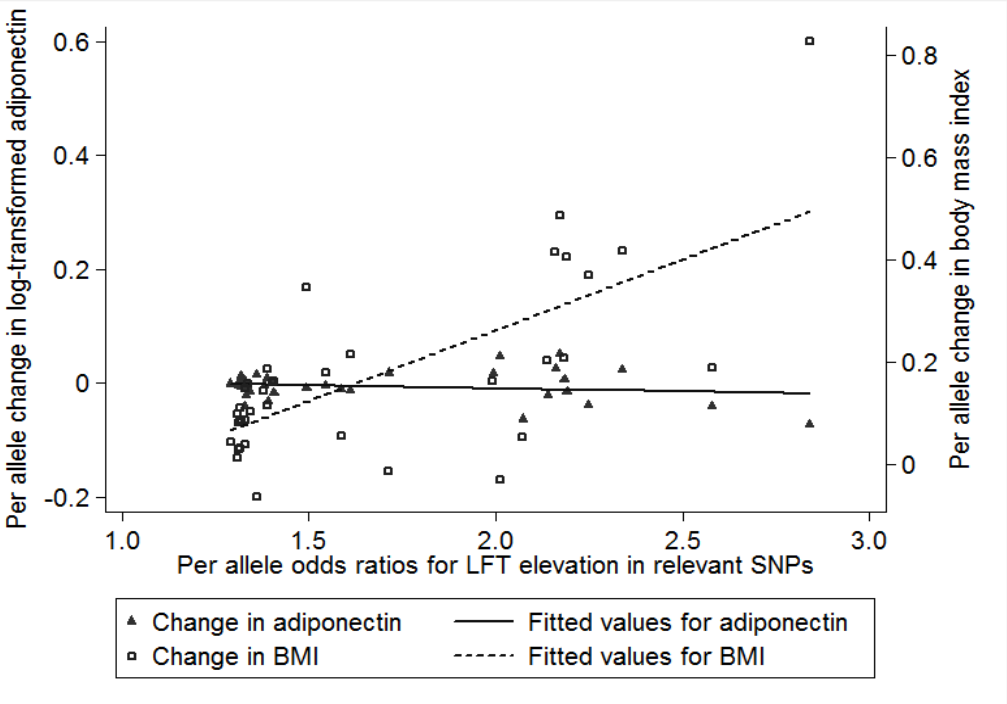


LFT, Liver function tests, i.e. serum aminotransferase (both of AST and ALT) levels; AST, Aspartate aminotransferase; ALT, Alanine transaminase

B3.

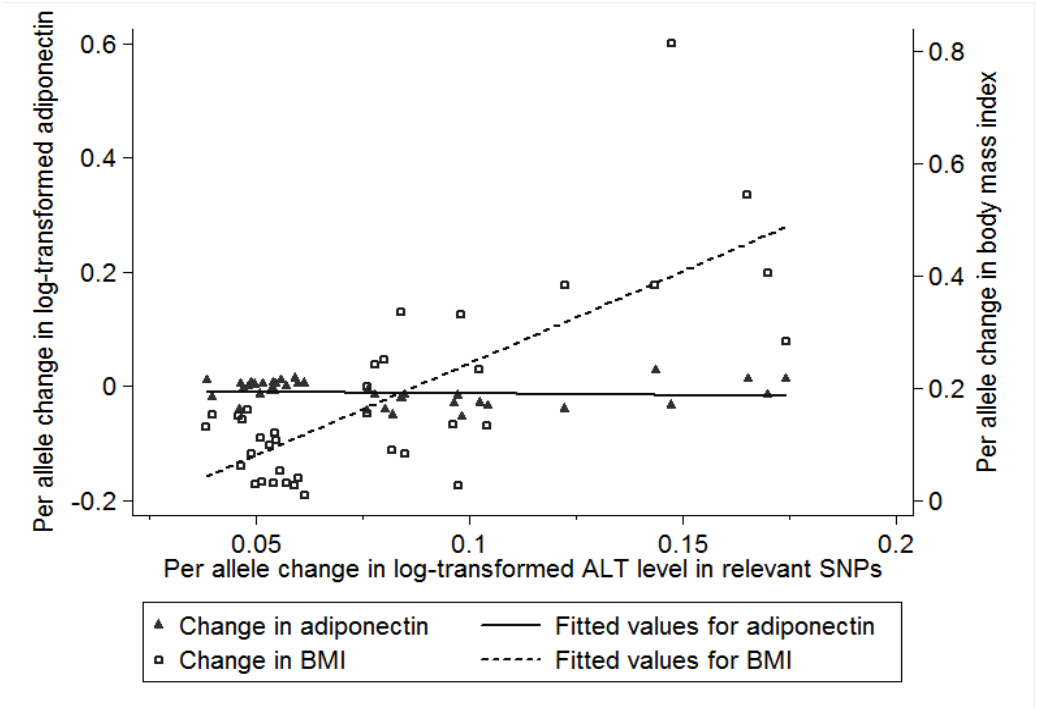
Reciprocal associations between outcome and exposure variables: effects (β coefficients) of (A) LFT elevation or (B) serum ALT level determining SNPs on serum adiponectin levels and body mass index

(A)



LFT, Liver function tests, i.e. serum aminotransferase (both of AST and ALT) levels; AST, Aspartate aminotransferase; ALT, Alanine transaminase

(B)



LFT, Liver function tests, i.e. serum aminotransferase (both of AST and ALT) levels; AST, Aspartate aminotransferase; ALT, Alanine transaminase

KOREAN ABSTRACT

Mendelian Randomization 분석을 이용한 아디포넥틴과 체질량지수, 혈청 아미노전이효소 간 인과성 추론

배경 및 연구목적

비만과 간 세포 손상 간의 관련성은 많은 연구들을 통해 밝혀져 왔으며, 아디포카인의 한 종류인 아디포넥틴은 간 세포에 대해서 지방질 축적 및 섬유화 또는 간 경화로부터의 보호 작용 가능성이 제기됨에 따라 다양한 연구의 대상이 되어 왔다. 그럼에도 불구하고, 관찰 연구의 한계로 인해 비만과 아디포넥틴, 그리고 간 질환 사이의 연관 관계에 대한 인과성은 입증될 수 없었다. 이 연구에서는 일반 인구집단 대상의 코호트 자료를 이용하여, 유전 형질을 도구변수로 활용한 Mendelian Randomization 방법을 통해 비만과 아디포넥틴 수치가 혈청 아미노전이효소 상승과 인과적 관련성을 가지는지를 평가하였다.

연구 대상과 방법

한국인 암 예방 연구 II (KCPS-II) 바이오뱅크의 정상인 서브코호트에 속한 혈청 아미노전이효소 수치 120IU/L 이하의 간염 바이러스 검사 음성 판정자 3,793 명을 대상으로, 아디포넥틴 수치 및 체질량지수에 대한 전장유전체 연관분석(Genome-wide association study, GWAS)을 시행하였다. 노출변수, 즉 중간 표현형과 높은 연관성을 보인 단일염기다형성(single nucleotide polymorphism, SNP) 혹은 가중치를 부여하여 통합한 유전위험점수(weighted

genetic risk score)가 Mendelian randomization 분석을 위한 도구변수로 사용되었다. 간 효소 수치 상승에 대한 노출변수의 인과적 영향력은 Wald 방법과 2 단계 최소자승법(two-stage least squares, 2SLS)을 통해 측정되었다.

연구 결과

CDH13 유전자의 rs4783244 와 그 외 7 개의 상호 독립적인 SNP 가 아디포넥틴 수치와 높은 연관성을 보이는 유전 도구변수로 선정되었으며, *BDNF* 유전자의 rs2030323 을 포함한 13 개의 독립적인 SNP 가 체질량지수에 대한 유전 도구변수로 선정되었다. 분석에 사용된 단일염기다형성과 유전위험점수는, 각 중간 표현형과의 강한 관련성 및 높은 설명력을 보여주었다. 관찰연구에서의 전통적 분석 방법에서는 유의하였던 아디포넥틴 수치와 간 효소 상승 간 연관성은 Mendelian randomization 분석에서는 유의하지 않은 결과를 보인 반면, 체질량지수로 표현된 비만과 간 효소 상승과의 관련성은 Mendelian randomization 분석을 통해서도 유의하게 입증되어 두 요소 간의 인과성을 지지하였다.

결론

본 연구는 Mendelian randomization 분석을 이용한 인과성 추론을 통해, 체내 과도한 지방이 간 세포 손상을 초래하는 원인으로 작용하며, 낮은 아디포넥틴 수치는 원인이 되기보다는 이러한 과정에서 나타나는 중간 결과임을 제시하였다. 이는 비만 혹은 대사증후군과 관련된 간 손상 또는 간질환의 예방과 치료에 있어서, 일차적 목표는 아디포넥틴의 증가가 아닌 체지방의 감소가 되어야 함을 시사한다는 점에서 그 의의를 지닌다.

핵심어: 아디포넥틴, 체질량지수, 간기능검사, Mendelian Randomization 분석, 유전위험점수