의학박사학위논문

비만과 고혈압의 인과성 연구: 멘델 무작위 및 유전-환경 상호작용 분석

Causal effects of obesity on hypertension: a Mendelian Randomization and gene-environment interaction analysis

2017 년 8 월

서울대학교 대학원

의학과 예방의학 전공

이 미 리

A thesis of the Doctor of Philosophy

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August 2017

The Department of Preventive Medicine,

Seoul National University

College of Medicine

Mee–ri Lee

ABSTRACT

Causal effects of obesity on hypertension: a Mendelian Randomization and gene–environment interaction analysis

Mee-ri Lee

Department of Preventive Medicine

The Graduate School

Seoul National University

Introduction: Hypertension is a risk factor for cardiovascular disease, and the burden of this disease gradually increased from 1990 and 2010. In 2014, 22% of people over the age of 18 worldwide were diagnosed with hypertension. Although previous observational studies have shown that obesity is a major risk factor for hypertension, unmeasured confounding factors or reverse causation may exist. In addition, the randomized controlled trials have had limitations because of short study periods or small

numbers of subjects. Therefore, Mendelian Randomization (MR) is necessary to prove causality.

Genome-wide association studies have reported that some genetic variants are related to hypertension, but genetic contributions to the development of hypertension have been reported to be low, i.e., less than 3%. It is important to reveal the candidate single nucleotide polymorphism (SNP) -obesity relationship to address this low accountability of genetic polymorphisms and identify groups with genetic susceptibility.

The aim of this study was to use MR to assess the causal effect of obesity on hypertension. Second, we analyzed the gene-obesity interaction for hypertension.

Methods: First, the MR analysis was performed in a well-defined community cohort study of 8832 adults (40-69 years) in Ansung and Ansan enrolled from 2001 to 2013. We used baseline hypertension and newly diagnosed hypertension during the 10-year follow-up period as the outcome variable. Genetic risk score associated with body mass index (BMI GRS) was used as the instrumental variable (IV) to measure the causal relationship between obesity and hypertension. The IV estimate of the causal

odds ratio (OR) was derived using the Wald ratio estimator and then exponentiation was used to express the result as an OR. The IV estimate of the causal hazard ratio (HR) was derived using the Wald ratio estimator and then exponentiation was used to express the result as a HR.

Second, in the interaction study, we used non-hypertensive subjects at baseline and for obesity variables, BMI, waist-to-hip ratio (WHR), and waist circumference (WC). We selected 3608 SNPs related to the pathway between obesity and hypertension and performed one degree-of-freedom (1df) and two degree-of-freedom (2df) tests for the interaction.

Results: The odds ratios (OR) with 95% confidence intervals for hypertension in an MR study using a multivariable model adjusting for age, sex, study area, education, smoking and current alcohol consumption was 1.19 (95% confidence interval (CI): 1.17-1.21) per unit increase in body mass index. We selected 6 SNPs (P-value $<1.0\times10^{-5}$) associated with BMI by genome-wide screening using linear regression and created six types of genetic risk score (GRS). We demonstrated that each standard-deviation increase in BMI GRS was associated with an OR for hypertension of 1.06-1.07 (all

P-values <0.05). Using BMI GRS as the IV, we found a causal relationship between BMI and hypertension (OR: 1.16-1.30, all P-values <0.05). Sensitivity analysis showed causality for baseline hypertension but not for incident hypertension.

Second, in the interaction study, we found 4 significant interactions (WHR and the SNPs rs6020611 and rs754118 in PTPN1; WC and rs3817588 in GCKR, and rs1864815 in ABCG5) for the development of hypertension (1df $P<0.01$, 2df $P<2 \times 10^{-6}$). We calculated GRS by summing the values of significant SNPs. The increment in the contributory proportions of BMI, WC, and WHR that explained hypertension, from the lowest to the highest weighted GRS, were 0.90%, 3.82%, and 2.65%, respectively, which were higher than the contributory proportions of GRS.

Conclusions: Using Mendelian randomization, we found that obesity is causally associated with hypertension. This information will have important public health implications, supporting evidence that obesity-reduction programs will reduce the incidence of hypertension. Also, we found that certain loci of the genes significantly interacted with obesity in the development of hypertension. Our study demonstrated that genetic predispositions

contribute to the development of hypertension more by the interaction with obesity than SNP effects themselves.

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keywords: Mendelian Randomization Analysis, gene–environment interaction, hypertension, obesity

Student Number: 2012–31149

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LIST OF ABBREVIATIONS

BMI, body mass index

CGRS, count genetic risk score

CI, confidence interval

DBP, diastolic blood pressure

GRS, genetic risk score

GWAS, genome-wide association studies

HR, hazard ratio

IV, instrumental variable

int., interaction effect

Imp, imputed

KoGES, Korean Genome and Epidemiology Study

MAF, minor allele frequency

MR, Mendelian randomization

OR, odds ratio

1df, one degree-of-freedom

RAAS, renin- angiotensin- aldosterone system

RCTs, Randomized controlled trials

SBP, systolic blood pressure

SD, standard deviation

SE, standard error

SNP single nucleotide polymorphism

2df, two degrees-of-freedom

WC, waist circumference

WGRS, weighted genetic risk score

WHR, waist-to-hip ratio

Ⅰ. Introduction

1. Hypertension and Obesity worldwide and in Korea

Hypertension is a major risk factor for ischemic heart disease, stroke, and chronic kidney disease. The global burden of these diseases increased substantially between 1990 and 2010. [1] In 2015, the global age-standardized mean systolic blood pressure was 127.0 mm Hg (95% confidence interval (CI) 125.7-128.3) in men and 122.3 mm Hg (121.0-123.6) in women; the global age-standardized mean diastolic blood pressure was 78.7 mm Hg (77.9-79.5) for men and 76.7 mm Hg (75.9-77.6) for women [2]; and the global age-standardized prevalence of hypertension was 24.1% (21.4-27.1) in men and 20.1% (17.8-22.5) in women. The highest age-standardized prevalence surpassed 35% in men in some countries in central and eastern Europe; prevalence was higher than 33% in women in a few countries in west Africa. [2] Korea belonged to the group of countries with low prevalence of hypertension, such as Canada, the United States, Peru, the United Kingdom, and Singapore, with an age-standardized prevalence of less than 13% in women and less than 19%

in men. [2] An increasing prevalence of hypertension and its related burdens is currently one of the main public health concerns in Korea.

Globally, the proportion of adults with a body-mass index (BMI) of 25 kg/m^2 or greater increased between 1980 and 2013 from 28.8% (95% CI 28.4–29.3) to 36.9% (36.3–37.4) in men, and from 29.8% (29.3–30.2) to 38.0% (37.5–38.5) in women. [3] In 2014, more than 1.9 billion adults 18 years and older were overweight. Of these overweight adults, over 600 million were obese. In 2014, 39% of adults 18 years of age and older were overweight, and 13% were obese. In the Korea National Health and Nutrition Examination Survey, the age-standardized proportion of adults with a BMI of 25 kg/m^2 or greater increased between 1998 and 2015 from 25.1% to 39.7% in men and from 25.1% to 26.0% in women. [4]

In the recent National Health Insurance service study, the prevalence of morbid obesity (BMI≥30) increased, and the socioeconomic costs of morbid obesity increased to 726.2 billion KRW in 2013, which was 1.47 times the cost in 2009 (492 billion KRW). [5]

Obesity is a major risk factor for hypertension [6-8], accounting

for 65–75% of the risk for primary hypertension [9], making obesity–related hypertension a major health issue [10]. It is therefore important to identify and manage obese individuals who are at high risk for hypertension. In the Framingham study, weight loss of 6.8 kg or more over 4 years led to a 21% to 29% reduction in hypertension risk. [11] Chandra et al. showed that a higher BMI and visceral adiposity were significantly associated with incident hypertension in African–American participants. [12] Lee et al. observed that obesity is associated with an increased risk of hypertension in the Korean population, regardless of the presence of other elements of metabolic syndrome. [13] In one recent study, morbid obesity was associated with hypertension (relative risk: 3.13; CI: 3.058–3.202) [5], and another study found that a 1 standard deviation increase in BMI, waist circumference (WC), waist–to–hip ratio (WHR), or waist–to–height ratio (WHtR) was significantly related to incident hypertension (hazard ratios: 1.39, 1.50, 1.40 and 1.49 in men, and 1.31, 1.44, 1.35 and 1.48 in women, respectively) in the Korean population. [14] Therefore, a study of the causal relationship between obesity and hypertension in Korea is needed.

2. Background of Mendelian randomization

Conventional observational analyses cannot avoid unmeasured confounding and reverse causation, making it difficult to infer causality from the observed association. [15, 16]

Randomized controlled trials (RCTs) have demonstrated the effect of weight loss on blood pressure. [17] However, some RCTs have yielded mixed results. Tyson et al. found that the weight-gain group (more than 3%) and the weight-stable (within 3%) group both had increased systolic blood pressure (SBP) and that the SBP of these two groups were not significantly different. [18] Moreover, SBP was unchanged in the weight-loss group who lost 3% or more of their weight. Furthermore, most of the RCTs were short-term studies with small numbers of participants; therefore, the results may not be applicable to the general population and cannot address the long-term health effects of obesity. In addition, the intervention could also affect other pathways. For example, weight loss surgery (e.g., Roux-en-Y gastric bypass, laparoscopic adjustable gastric banding, or vertical sleeve gastrectomy) influences glucose metabolism more than it influences the obesity-hypertension pathway. [19]

Mendelian randomization (MR) analysis using genetic variants as the instrumental variable (IV) has been increasingly used to assess causality. Genetic variants are present from conception, allocated randomly according to Mendel's second law and are inherited independent of potential confounding factors. [15, 16] Thus, the IV (genetic variants associated with obesity) is independent of confounders in its effects on the phenotype (obesity) – outcome (hypertension) relationship.

Recently, a small number of MR studies have reported that BMI has a causal relationship with hypertension. [20–22] However, these studies were conducted in Western populations. The World Health Organization reported that the prevalence of overweight (BMI $\geq$25 kg/m^2) and obesity (BMI $\geq$30 kg/m^2) is highest in the Americas (61% overweight or obese in both sexes, and 27% obese), especially in the US (68% overweight or obese among both sexes, and 32% obese). In contrast, Koreans have a low prevalence of obesity (31% overweight or obese among both sexes and 4.6% obese). However, the prevalence of hypertension is similar between the US and Korea (9.4% vs. 8.4%, respectively). [23, 24] Because of the different prevalences

of obesity but similar prevalences of hypertension between the US and Korea, a study of the causal relationship between obesity and hypertension in Korea is needed.

3. Background of gene-environment interaction

The Framingham Heart Study and twin studies estimated that genetic factors represent one-third to one-half of the inter-individual variability of blood pressure values. [25, 26] However, genetic variants identified in the genome-wide association studies (GWAS) explain <3% of the blood pressure variability. [27] Recently, several studies have reported that some genetic polymorphisms interact with environmental factors to exert their effect on the BP [28-30]. Sung et al. [30] identified 7 significant and 21 suggestive BP loci for the SNP-smoking interaction, and Simino et al. [29] found that the effect of SNPs in the gene *SLC16A9* on SBP was significantly modulated by drinking alcohol. Basson et al. [28] reported that SNPs in *PTN* and *TOX2* were associated with an increased BP in those with less education. However, these previous studies carried out agnostic genome-wide analyses of interaction, including all SNPs in the analysis, and one limitation was that interpretation of the biological mechanism of the significant loci was difficult. Furthermore, previous studies used cross-sectional data for the effects of gene-environment interactions on BP measured at a single visit.

Obesity is a major risk factor for hypertension, accounting for 65–75% of the risk for primary hypertension [9], which make obesity–related hypertension a major health issue. [10] It is therefore important to identify and manage an obese group at high risk for hypertension.

Several pathogenic mechanisms have been suggested to contribute to the development of hypertension in an obese population: insulin resistance, vascular alterations, and activation of the renin–angiotensin–aldosterone system (RAAS). [31, 32] Excess adipose tissue stimulates insulin secretion, which activates the sympathetic nervous system (SNS) and raises the BP. [33] Insulin also acts directly on the kidneys to stimulate sodium retention, increase plasma volume, and raise the BP. [34] Vascular alterations, including structural changes, endothelial dysfunction, and altered stiffness are common in obesity and are also thought to contribute to the development of hypertension. [35, 36] An activated RAAS in the presence of the excess adipose tissue of obese people generates angiotensin and aldosterone, which again elevate the BP. [31]

Because genetic polymorphisms related to these mechanisms could modify the effect of obesity on the development of hypertension, the

study of pathway-related SNPs may enhance our understanding of the interaction between genes and obesity.

To our knowledge, there have been few reports regarding genetic variants modifying the relationship between obesity and hypertension. Xi et al. [37] selected six SNPs from an earlier GWAS of hypertension, calculated the genetic risk score (GRS), and observed a significant association of SNPs and GRS with hypertension in obese Chinese children, but not in children of normal weight. Ji et al. [38] found that an interaction between the SNP rs4305 on the *RAAS* genes and BMI increased the susceptibility to hypertension in a case-control study of Han Chinese individuals. Kim et al. [39] reported an interaction between the SNP rs13390641 on 2q12.1 and BMI affecting the SBP in Korean and Japanese populations. However, these studies used cross-sectional data that had limitations in the evaluation of the development of hypertension.

Ⅱ. Mendelian Randomization study

1. Study aim and Hypotheses

1.1. Study aim

In the Mendelian Randomization study, our aim was to analyze the association between the IV for obesity using the BMI-associated genetic risk score (BMI GRS) and the risk of hypertension to explore the causal association between obesity and hypertension, because a composite genetic risk score (GRS) reduces the statistical error associated with multiple testing compared to individual SNPs. Figure 1 shows the directed acyclic graphs between exposure (BMI) and outcome (hypertension) with the genetic instrument.

1.2. Hypotheses

Hypothesis 1: Obesity has a causal relationship with hypertension when considering both baseline hypertension and newly diagnosed hypertension during the 10-year follow-up period.

Hypothesis 2: Obesity has a causal relationship with the prevalence of hypertension.

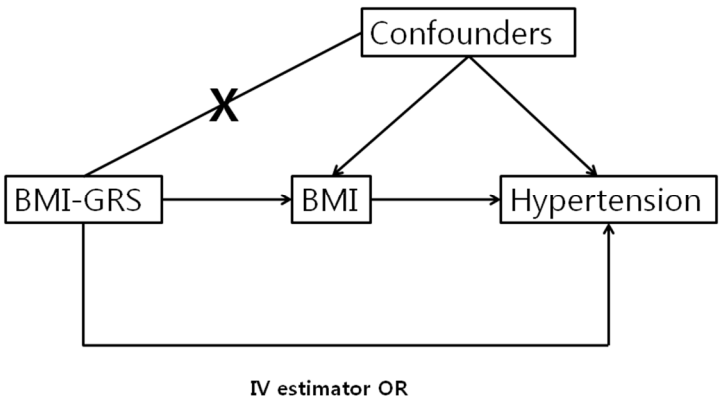

Figure 1. Directed acyclic graph explaining the relationships between exposure (BMI) and outcome (hypertension) with the genetic instrument (genetic score).

BMI, body mass index; CGRS, count genetic risk score; IV, instrumental variable; OR, odds ratio; SD, standard deviation; WGRS, weighted genetic risk score

2. Materials and methods

2.1. Study population

We used data from the Ansung-Ansan cohort within the Korean Genome and Epidemiology Study (KoGES), which was initiated in 2001 as a population-based cohort study recruiting Korean adults aged 40-69 years. Briefly, a total of 5020 participants (2523 men and 2497 women) in Ansan and 5018 participants (2239 men and 2779 women) in Ansung were included in the baseline examinations from June 2001 to January 2003. Follow-up surveys were conducted biennially, and study participants were followed-up up to five times until 2012. Information about their general characteristics, lifestyle, and current medications was obtained through questionnaires. Physical examinations, including BP, anthropometric measurements, and blood sampling were conducted by trained researchers from 2001 to 2012. During this 10-year period, a follow-up rate of 62.1% was achieved.

The criteria for exclusion were missing BP measurements or history of hypertension diagnosis; we excluded 10 participants. The present report focuses on 8832 participants for whom information

about genotype and the outcome variable of hypertension was available (Figure 2).

An informed consent form was signed by each participant, and the study protocol was approved by the institutional review board of the Seoul National University Hospital (IRB No. 1312-033-539)

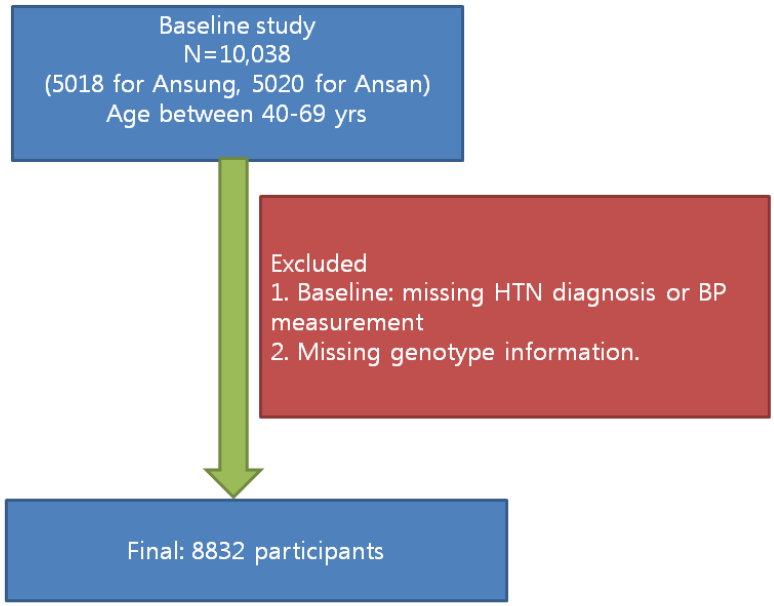

Figure 2. Study subjects for MR study.

2.2. Genotypes

A total of 10,004 participants were genotyped using the Affymetrix Genome-Wide Human SNP Array 5.0 (Santa Clara, CA, USA) containing 500,568 SNPs. Genotype clustering was determined using Bayesian robust linear modeling of the Mahalanobis distance. Before statistical analysis, 17,926 markers with a genotype call rate <95%, 92,050 markers with low minor allele frequency (<0.01), and 38,364 markers with Hardy-Weinberg equilibrium (P-value $<10^{-6}$) were removed, leaving 352,228 SNPs for 8842 individuals. An additional 1.8×10^{6} SNPs were found by imputation using the JPT/CHB component of HapMap as the reference. After filtering, a total of 1,590,162 genotyped and imputed SNPs were available for analysis. The genotyping methods of the KoGES have been described in detail previously. [40]

2.3. Obesity and covariates

The BMI was calculated as weight in kilograms divided by height in meters squared (kg/m^2) at the baseline survey.

Alcohol consumption was calculated as the amount consumed per week and divided into two groups. Based on the guidelines for recommended alcohol consumption to lower health risks from the Korea Health Promotion Foundation, we defined low consumption of alcohol as 40 g or less for males and 20 g or less for females at one time, less than twice a week. [41] They were also split into two groups by smoking status: less than 20 pack-years smoking and greater than 20 pack-years.

We used BMI (kg/m^2), age (years), sex (male, female), area (Ansung, Ansan), education (≤ 9 or >9 years of school), alcohol consumption and smoking from the baseline survey.

2.4. Hypertension assessment

BP was measured using mercury sphygmomanometers (Baumanometer; WA Baum, Copiague, NY, USA) according to a standardized protocol. [42] All measurements in the present study were taken after at least a 5-min rest. We used the average of three measurements. At baseline, hypertensive participants were defined as having SBP ≥140 mmHg or DBP ≥90 mmHg, using antihypertensive medication, or having a history of hypertension diagnosed by a doctor. After these participants were excluded, newly diagnosed cases of hypertension were defined as SBP ≥140 or DBP ≥90 mmHg and taking antihypertensive drugs during the 10-year follow-up. We considered both baseline hypertension and newly diagnosed hypertension during the 10-year follow-up period. In the baseline study, where information about the use of blood pressure lowering medication was available, a constant was added to SBP (15 mm Hg) and DBP (10 mm Hg) in subjects on blood pressure lowering medication, as recommended by Tobin et al. [43] If this information was not available, SBP and DBP were analyzed as they were.

2.5. Statistical analysis

Multivariate logistic regression models were used to assess the association between BMI and hypertension. Model 1 was not adjusted for other variables; Model 2 was adjusted for age (years) and sex (male or female); Model 3 was further adjusted for region (Ansung or Ansan), education (≤9 or >9 years of school), tobacco smoking, and current alcohol consumption. The association between BMI GRS and hypertension was evaluated in a bivariate logistic regression model.

In MR analysis, we used the six types of BMI GRS as the IV estimators to measure the strength of the causal relationship between BMI and hypertension. The IV estimate of causal odds ratio (OR) was derived using the Wald-type estimator and then exponentiation to express the result as an OR. [20] $OR_{GRS-hypertension}$ estimated the effect of the GRS on hypertension using univariate logistic regression. $\beta_{GRS-BMI}$ estimated the effect of the GRS on BMI using linear regression.

$$OR_{IV} = \exp\left(\frac{Ln\left(OR_{GRS-hypertension}\right)}{\beta_{GRS-BMI}}\right)$$

We also tested the difference between the IV estimators and the conventional regression-based estimators for the effect of BMI using

a classical z-test.

In the sensitivity analysis, we conducted MR analysis using only baseline data for a cross-sectional approach and using only incident data for Cox proportional hazards regression analysis. We also conducted the MR study using relative risk. A causal hazard ratio for the association of BMI with incident hypertension was derived using the Wald-type estimator with standard errors estimated by the delta method.

$$\text{Causal HR} = \text{Exp}(\log(\text{HR}_{\text{GRS-incident hypertension}})/\beta_{\text{GRS-BMI}})$$

A computerized literature review was conducted to identify articles published before June 2017 using PubMed, Embase, and the Cochrane Library. The search terms used were: (overweight) OR (obesity) OR (adiposity) OR (body mass index) OR (BMI) OR (intra-abdominal fat) OR (waist hip ratio) OR (waist circumference)) AND ((blood pressure) OR (hypertension)) AND (mendelian randomization analysis). The search was run according to Medical Subject Headings (MeSH) without restriction to regions or publication types. The language was restricted to English. For the MR study of a continuous

variable (blood pressure), we used the "ivregress 2sls" command in the Stata software package (version 12.0; Stata Corp., College Station, TX, USA). Meta-analyses were carried out using the Stata "metan" command. Heterogeneity was assessed by calculating I^2. I^2>50% was considered to signify significant heterogeneity. Meta-analyses for the binary hypertension variable was conducted using a fix effects model. Meta-analysis for a continuous variable (blood pressure) was conducted using a random effects model due to substantial heterogeneity (I^2> 50%).

Statistical significance was set to a two-sided *P*-value of less than 0.05. All statistical analyses were performed using SAS software version 9.3 (SAS Institute Inc., Cary, NC, USA) and R version 3.1.0 (Comprehensive R Archive Network: http://cran.r-project.org). PLINK (version 1.08, http://pngu.mgh.harvard.edu/~purcell/plink) was used to extract relevant SNPs from the raw genotype data of the Ansung-Ansan populations from both genotyped and imputed sequencing datasets and to calculate the rare allele frequency (RAF). Haploview (http://www.broadinstitute.org/haploview/haploview) was used to test for linkage disequilibrium of the extracted SNPs.

3. Results

3.1. Selection of genetic loci and GRS construction

We performed linear regression and found 32 individual SNPs associated with BMI (Table 1). Because we wanted to include more SNPs, we used a liberal *P*-value ($<1.0\times10^{-5}$) instead of a restrictive *P*-value after the Bonferroni correction, 5.0×10^{-8}. Among these SNPs, two SNPs had been reported previously. [40] Some SNPs were found to be in high linkage disequilibrium ($|D'|\geq0.9$; see Figure 3). Therefore, we selected one representative SNP from the closely linked SNPs based on the estimated size of the main genetic analysis results or significance in previous studies. Finally, three BMI GRSs were constructed. The first BMI GRS was composed of 2 significant SNPs (rs17178527 and rs9939609) found in a previous study. [40] The second was composed of 4 SNPs (rs17178527, rs9939609, rs7668087, and rs11000212) selected with a cut off *P*-value $<5x10^{-6}$. The third was composed of 6 SNPs (rs17178527, rs9939609, rs7668087, rs11000212, rs17130257, and rs10936246) selected with a cut off *P*-value $<5x10^{-5}$. The GRS was produced by two methods: a simple count method (CGRS) and a weighted method

(WGRS). [44, 45] Six types (3x2) of BMI GRS (CGRS (n=2), WGRS (n=2), CGRS (n=4), WGRS (n=4), CGRS (n=6), and WGRS (n=6)) were used in the analysis. We assumed an additive genetic model for each SNP, applying a linear weighting of 0, 1, or 2 to genotypes containing 0, 1, or 2 risk alleles, respectively. The simple count model assumes that each SNP in the panel contributes equally to the risk of hypertension and was calculated by summing the values (0, 1, and 2) for each of the SNPs. The weighted GRS was calculated by multiplying each β coefficient obtained from linear regression by the number of corresponding risk alleles (0, 1, and 2). All β coefficients were positive because we reordered the sequence of genotypes of the SNPs when the weights were less than zero.

Table 1. Genome-wide association values for BMI (P-value $<10^{-5}$).

CHR	Position	Gene	SNP	Minor	Major	MAF	BETA	SE	P-value
1	88104694	NID	rs17130257	C	T	0.06961	-0.407	0.091	8.18E-06
3	161803575	NID	rs10936246	A	G	0.06912	0.413	0.093	9.62E-06
3	161804954	NID	rs1436740	C	T	0.06902	0.413	0.093	9.79E-06
3	161805098	NID	rs4273381	T	A	0.06902	0.413	0.093	9.79E-06
3	161805155	NID	rs1436739	C	T	0.06902	0.413	0.093	9.79E-06
4	36340970	DTHD1	rs7668087	A	G	0.08796	0.4	0.087	4.13E-06
6	141584943	NID	rs17178527	A	G	0.2486	-0.31	0.055	1.95E-08
6	141671488	NID	rs7770810	G	A	0.2414	-0.311	0.055	2.13E-08

6	141673218	NID	rs1577948	G	A	0.2415	−0.31	0.055	2.25E−08
6	141674235	NID	rs1572604	T	C	0.2415	−0.31	0.055	2.25E−08
6	141674807	NID	rs1572605	G	C	0.2416	−0.31	0.055	2.36E−08
6	141703203	NID	rs17054002	T	C	0.239	−0.296	0.056	1.45E−07
10	72123792	ASCC1	rs1245579	T	C	0.1917	0.277	0.06	4.46E−06
10	72146392	ASCC1	rs1668157	G	A	0.1911	0.277	0.061	5.34E−06
10	72195894	ASCC1	rs11000212	G	C	0.2057	0.284	0.058	1.01E−06
16	53769662	FTO	rs1558902	A	T	0.1259	0.338	0.07	1.65E−06
16	53776774	FTO	rs7193144	C	T	0.1256	0.332	0.07	2.50E−06
16	53779455	FTO	rs17817449	G	T	0.126	0.33	0.07	2.74E−06
16	53779538	FTO	rs8043757	T	A	0.126	0.33	0.07	2.74E−06

16	53782363	FTO	rs8050136	A	C	0.1262	0.329	0.07	2.69E-06
16	53782840	FTO	rs8051591	G	A	0.1259	0.331	0.07	2.65E-06
16	53782926	FTO	rs9935401	A	G	0.1259	0.331	0.07	2.65E-06
16	53784548	FTO	rs3751812	T	G	0.1262	0.331	0.07	2.52E-06
16	53785257	FTO	rs9936385	C	T	0.1279	0.328	0.071	3.34E-06
16	53785965	FTO	rs11075989	T	C	0.1262	0.331	0.07	2.52E-06
16	53785981	FTO	rs11075990	G	A	0.1262	0.331	0.07	2.52E-06
16	53786591	FTO	rs9926289	A	G	0.1265	0.327	0.07	3.24E-06
16	53786615	FTO	rs9939609	A	T	0.1262	0.337	0.07	1.72E-06
16	53787213	FTO	rs17817712	G	A	0.1218	0.348	0.072	1.27E-06
16	53787703	FTO	rs7202116	G	A	0.1253	0.338	0.071	2.16E-06

16	53788739	FTO	rs7185735	G	A	0.1256	0.335	0.071	2.67E−06
16	53794154	FTO	rs17817964	T	C	0.1298	0.331	0.072	4.91E−06

BMI, body mass index; CHR, chromosome; Minor, minor allele; NID: Not identified; SE, standard error;

SNP, single nucleotide polymorphism

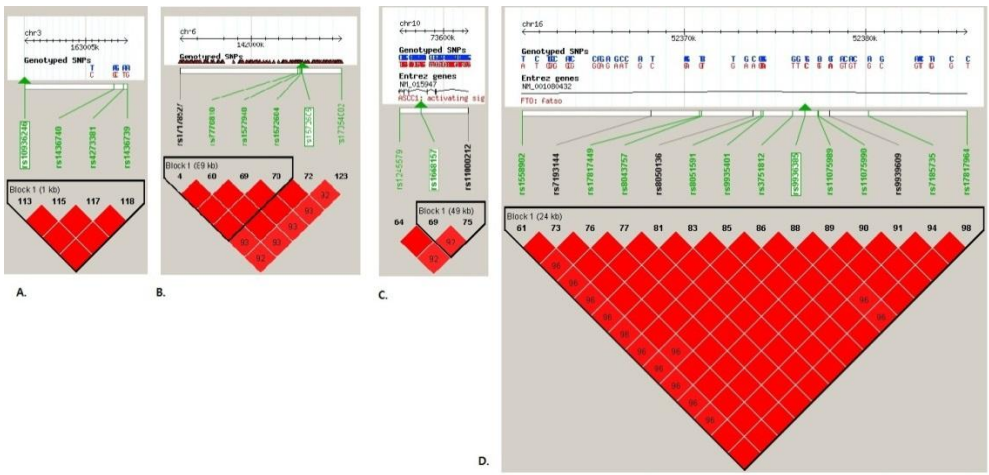

Figure 3. Linkage-disequilibrium plot for SNPs (A: chromosome(chr) 3; B: chr6; C: chr10; D: chr16).

3.2. Study cohorts

Among 8832 participants, 4179 (47.3%) were men. The average age was 52 (SD 8.92) years, and the average BMI was 24.6 (SD 3.12)kg/m^2. At baseline, hypertension was diagnosed in 2971 (33.6%) participants, and the remaining 5861 (66.4%) participants were not hypertensive. During the 10-year follow-up, hypertension was newly detected in 1409 participants (first follow-up: 436; second follow-up: 274; third follow-up: 232; fourth follow-up: 322; and fifth follow-up: 145). The number (proportion) of hypertensive participants (baseline and new hypertension) was 4380 (49.6%) (Figure 4). As shown in Table 2, there were statistically significant differences in age, area, education, alcohol consumption and BMI measured between the hypertensive and non-hypertensive groups.

Table 2. General characteristics of the study population (n=8832).

Variable		No HTN at baseline	HTN at baseline	*P*-value
Total no.		4452 (50.4)	4380 (49.6)	
Age (years)		50.2 (8.4)	56.1 (8.6)	<0.0001
Sex	Male	2061 (46.3)	2118 (48.4)	0.052
	Female	2391 (53.7)	2262 (51.6)	
Area	Ansung	1625 (36.5)	2576 (58.8)	<0.0001
	Ansan	2827 (63.5)	1804 (41.2)	
Education (years of school)	≤9	2077 (46.9)	2821 (65.1)	<0.0001
	>9	2350 (53.1)	1512 (34.9)	
	Missing	72		
Alcohol (grams)	Male: <40, female: <20	3169 (71.2)	3011 (68.7)	0.013
	Male: ≥40, female: ≥20	1283 (28.8)	1369 (31.3)	
Smoking	No	2627 (59.6)	2510 (58.3)	0.211
	Yes	1781 (40.4)	1797 (41.7)	

	Missing	117		
BMI (kg/m^2)	<25	2951 (66.3)	2082 (47.6)	<0.0001
	≥25	1500 (33.7)	2295 (52.4)	
	Missing	4		

*$\chi 2$ test and Student's t-test were used for categorical and continuous variables, respectively.

BMI, body mass index

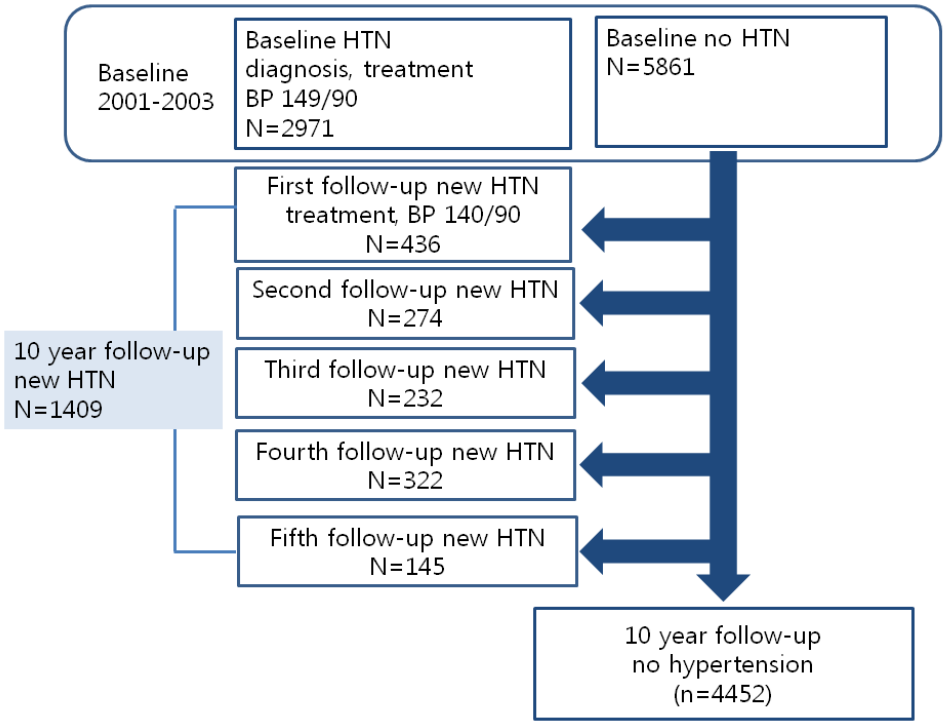

Figure 4. Flow chart of study cohort.

3.3. BMI and hypertension

To visualize a linear relationship between the continuous variables of BMI and blood pressure, we used generalized additive mixed models of R (package 'gamm4') with cubic smoothing spline (k=3). We used BMI and blood pressure at every visit. There was a linear relationship between BMI and blood pressure using the generalized additive mixed model (Figure 5).

Table 3 shows the demographic features of the participants according to BMI GRS quartiles. The BMI GRS (in quartiles) was significantly associated with BMI (*P*-value for trend <0.0001). No other population characteristics (sex, area, smoking, current alcohol drinking) were associated with the BMI GRS (n=6) quartiles (all *P*-values for trend >0.05).

As shown in Table 4, in the multivariable adjusted model, the odds ratio (OR) with 95% confidence intervals for hypertension was 1.19 (95% confidence interval (CI): 1.17-1.21) per unit increase in body mass index. Each SD increase in BMI GRS was associated with an OR for hypertension of 1.06-1.07 (all P-values <0.05).

The association between BMI-GRS and BMI is shown in Table 5.

Figure 6 shows the MR results. In the IV analysis, the causal OR of a 1 kg/m^2 increase in BMI for hypertension was 1.16-1.30 (all *P*-values <0.05). Compared to the IV using GRS (n=4 or 6), IV using GRS (n=2) yielded a greater OR in MR analysis. The causal estimate of the relationship between BMI and hypertension risk using the IV variable and the observed association between BMI and hypertension risk were not significantly different in a classical z-test (1.16-1.30 vs. 1.19, *P*-value >0.05).

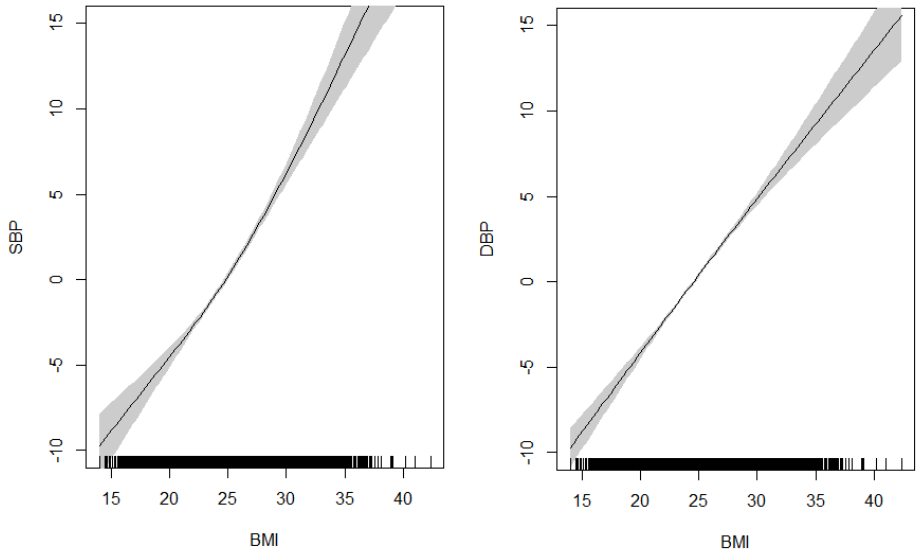

Figure 5. Generalized additive mixed model for BMI, SBP and DBP.

Table 3. Characteristics of study participants according to the weighted BMI genetic risk score (BMI GRS) (n=6).

Characteristic	Quartile 1 (n=1863)	Quartile 2 (n=1929)	Quartile 3 (n=1575)	Quartile 4 (n=2277)	*P* for trend
BMI GRS	1.05 (0.18)	1.43 (0.02)	1.64 (0.11)	2.01 (0.24)	<0.0001
BMI, kg/m^2	24.09 (2.98)	24.51 (3.06)	24.76 (3.17)	25.07 (3.18)	<0.0001
Age, years	52.54 (8.97)	52.20 (9.04)	52.09 (8.86)	52.20 (8.86)	0.23
Male, n (%)	892 (47.9)	903 (46.8)	745 (47.3)	1071 (47.04)	0.688
Live in Ansan, n (%)	953 (51.2)	1032 (53.5)	814 (51.7)	1221 (53.6)	0.237
Education (years) >9	804 (43.6)	857 (44.7)	665 (42.6)	1019 (45.2)	0.51
Smoking, n (%)	776 (42.2)	788 (41.5)	638 (40.9)	902 (40.2)	0.178
Current drinking, n (%)	556 (29.8)	571 (29.6)	484 (30.7)	681 (29.9)	0.82

Quartile: Quartile 1 (<1.36), Quartile 2 (≥1.36, <1.46), Quartile 3 (≥1.46, <1.77), Quartile 4 (≥1.77)

Table 4. The association of BMI GRS and BMI with hypertension.

		Model 1	Model 2	Model 3
	SD	OR (95% CI)	OR (95% CI)	OR (95% CI)
BMI GRS, per SD				
CGRS (n=2)	0.77	1.06 (1.02−1.11)		
WGRS (n=2)	0.25	1.07 (1.02−1.11)		
CGRS (n=4)	1.04	1.07 (1.02−1.12)		
WGRS (n=4)	0.34	1.07 (1.02−1.12)		
CGRS (n=6)	1.15	1.06 (1.02−1.11)		
WGRS (n=6)	0.39	1.06 (1.01−1.11)		
BMI (kg/m^2)		1.15 (1.13−1.16)	1.19 (1.17−1.20)	1.19 (1.17−1.21)

Data are presented as odds ratio (OR) and 95% confidence interval (CI).

Model 1 was not adjusted for other variables. Model 2 was adjusted for age (years) and sex (male and female). Model 3 was further adjusted for area (Ansung and Ansan), education (≤9 and >9 years of school), smoking and current alcohol consumption. BMI, body mass index; CGRS, count genetic risk score; SD, standard deviation; WGRS, weighted genetic risk score

Table 5. The association of BMI-GRS with BMI (per SD).

	SD	beta	95% CI	p-value
CGRS	0.77	0.24	0.18-0.31	<.0001
WGRS	0.25	0.24	0.18-0.31	<.0001
CGRS	1.04	0.33	0.26-0.40	<.0001
WGRS	0.34	0.33	0.26-0.40	<.0001
CGRS	1.15	0.38	0.31-0.45	<.0001
WGRS	0.39	0.38	0.31-0.45	<.0001

BMI, body mass index; CGRS, count genetic risk score; CI, confidence interval; SD, standard deviation; WGRS, weighted genetic risk score

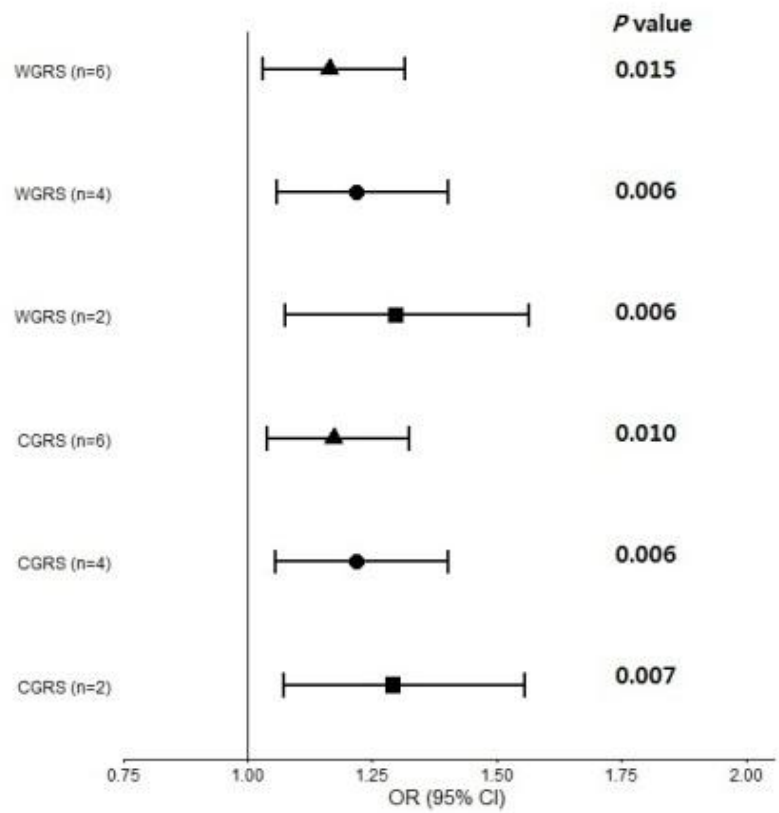

Figure 6. Instrumental variable (IV)−estimated association of BMI and hypertension (baseline and newly diagnosed hypertension).

BMI, body mass index; CGRS, count genetic risk score; CI, confidence interval; OR, odds ratio; SD, standard deviation; WGRS, weighted genetic risk score

3.4. Sensitivity analysis

We also conducted a sensitivity analysis with baseline hypertension only. Table 6 showed that the OR for baseline hypertension was 1.05−1.07 per SD increase in the six types of BMI GRS. The OR with 95% confidence intervals for baseline hypertension was 1.18 (95% confidence interval (CI): 1.17−1.20) per unit increase in body mass index.

In the IV analysis, BMI was found to have a causal relationship with baseline hypertension for the six types of GRS BMI. The causal OR of a 1 kg/m^2 increase in BMI for hypertension was 1.13−1.31 (all *P*−values <0.05 except WGRS (n=6)) (Figure 7), and there was no significant difference between IV analysis and multivariate analysis in a classical z−test (1.13−1.31 vs. 1.18, *P*−value >0.05).

We conducted a sensitivity analysis with incident hypertension only. Table 7 showed that all GRS were not significant with incident hypertension.

The HR with 95% confidence intervals for incident hypertension was 1.11 (95% confidence interval (CI): 1.09−1.13) per unit increase in

body mass index in the Cox proportional hazard model.

Table 6. The association of BMI GRS and BMI with baseline hypertension (No. of subjects= 8832, No. of events= 2971).

		Model 1	Model 2	Model 3
	SD	OR (95% CI)	OR (95% CI)	OR (95% CI)
BMI GRS, per SD				
CGRS (n=2)	0.77	1.07 (1.02−1.12)		
WGRS (n=2)	0.25	1.07 (1.02−1.12)		
CGRS (n=4)	1.04	1.06 (1.01−1.11)		
WGRS (n=4)	0.34	1.06 (1.01−1.11)		
CGRS (n=6)	1.15	1.05 (1.00−1.10)		
WGRS (n=6)	0.39	1.05 (1.00−1.10)		
BMI (kg/m^2)		1.15 (1.13−1.17)	1.18 (1.16−1.2)	1.18 (1.17−1.20)

Data are presented as odds ratio (OR) and 95% confidence interval (CI). Model 1 was not adjusted for other variables. Model 2 was adjusted for age (years) and sex (male and female). Model 3 was further adjusted for area (Ansung and Ansan), education (≤9 and >9 years of school), smoking and current alcohol consumption. BMI, body mass index; CGRS, count genetic risk score; SD, standard deviation; WGRS, weighted genetic risk score

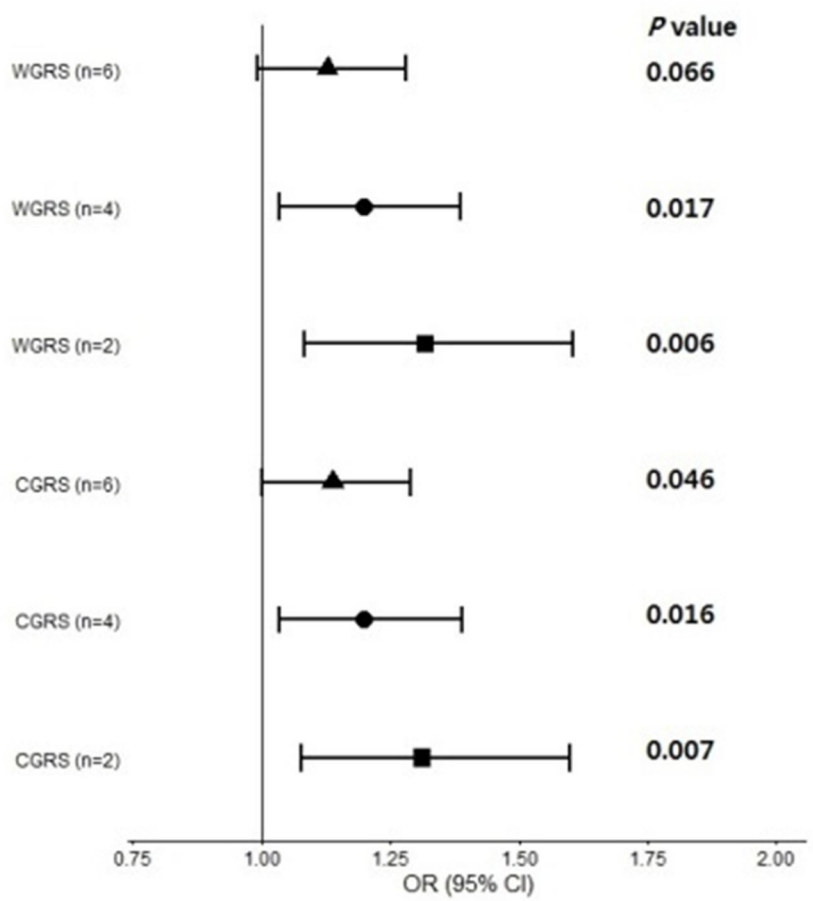

Figure 7. Instrumental variable (IV)–estimated association of BMI and baseline hypertension.

BMI, body mass index; CGRS, count genetic risk score; CI, confidence interval; OR, odds ratio; SD, standard deviation; WGRS, weighted genetic risk score

Table 7. The association of BMI GRS and BMI with incident hypertension.

		Model 1	Model 2	Model 3
	SD	HR (95% CI)	HR (95% CI)	HR (95% CI)
BMI GRS, per SD				
CGRS (n=2)	0.77	1.03 (0.97−1.08)		
WGRS (n=2)	0.25	1.03 (0.97−1.08)		
CGRS (n=4)	1.04	1.03 (0.98−1.09)		
WGRS (n=4)	0.34	1.03 (0.98−1.09)		
CGRS (n=6)	1.15	1.03 (0.99−1.10)		
WGRS (n=6)	0.39	1.04 (0.99−1.10)		
BMI (kg/m^2)		1.08 (1.06−1.10)	1.11 (1.09−1.12)	1.11 (1.09−1.13)

Data are presented as hazard ratio (HR) and 95% confidence interval (CI).

Model 1 was not adjusted for other variables. Model 2 was adjusted for age (years) and sex (male and female). Model 3 was further adjusted for area (Ansung and Ansan), education (≤9 and >9 years of school), smoking and current alcohol consumption. BMI, body mass index; CGRS, count genetic risk score; SD, standard deviation; WGRS, weighted genetic risk score

We conducted sensitivity analyses using relative risk.

As shown in Table 8, in the multivariable adjusted model, the RR with 95% confidence intervals for hypertension was 1.01 (95% confidence interval (CI): 1.008−1.012) per unit increase in body mass index. The RR with 95% confidence intervals for hypertension was 1.03 per SD increase in the six types of BMI−GRS.

Figure 8 shows the MR results. In the IV analysis, the causal RR of a 1 kg/m^2 increase in BMI for hypertension was 1.08−1.14 (all *P*−values <0.05). Compared to the IV using GRS (n=4 or 6), IV using GRS (n=2) yielded a greater RR in MR analysis. The causal estimate of the relationship between BMI and hypertension risk using the IV variable and the observed association between BMI and hypertension risk were significantly different in a classical z−test (1.08 −1.14 vs. 1.01, *P*−value <0.05).

We conducted the MR study using the continuous measurement of baseline blood pressure. (Table 9) BMI had a causal relationship with SBP only in CGRS (n=2).

Table 8. The association of BMI GRS and BMI with hypertension.

		Model 1	Model 2	Model 3
	SD	RR (95% CI)	RR (95% CI)	RR (95% CI)
BMI GRS, per SD				
CGRS (n=2)	0.77	1.03 (1.01−1.05)		
WGRS (n=2)	0.25	1.03 (1.01−1.05)		
CGRS (n=4)	1.04	1.03 (1.01−1.06)		
WGRS (n=4)	0.34	1.03 (1.01−1.06)		
CGRS (n=6)	1.15	1.03 (1.01−1.05)		
WGRS (n=6)	0.39	1.03 (1.01−1.05)		
BMI (kg/m^2)		1.02 (1.01−1.02)	1.01 (1.01−1.01)	1.01 (1.01−1.01)

Data are presented as relative risk (RR) and 95% confidence interval (CI).

Model 1 was not adjusted for other variables. Model 2 was adjusted for age (years) and sex (male and female). Model 3 was further adjusted for area (Ansung and Ansan), education (≤9 and >9 years of school), smoking and current alcohol consumption. BMI, body mass index; CGRS, count genetic risk score; SD, standard deviation; WGRS, weighted genetic risk score

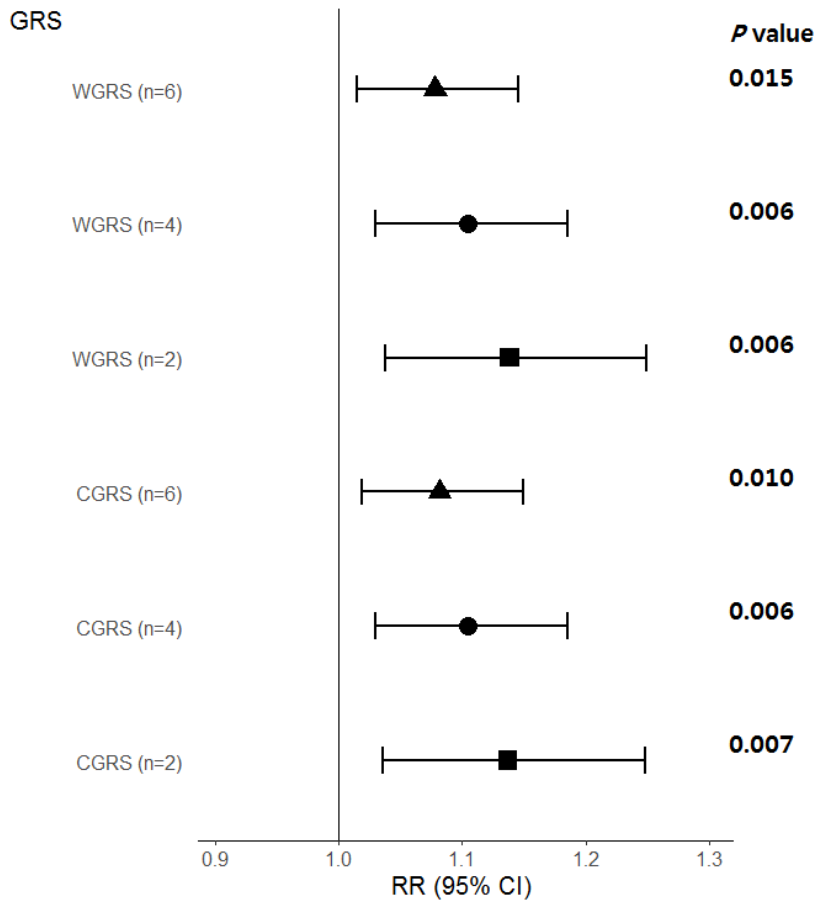

Figure 8. Instrumental variable (IV)–estimated association of BMI and hypertension (baseline and newly diagnosed hypertension).

BMI, body mass index; CGRS, count genetic risk score; CI, confidence interval; RR, relative ratio; SD, standard deviation; WGRS, weighted genetic risk score

Table 9. MR study using continuous measurement of baseline blood pressure.

GRS	Beta	SD	*p*	95% LCI	95% UCI
SBP					
CGRS(n=2)	0.0055	0.0027	0.0410	0.0002	0.0108
WGRS(n=2)	0.0003	0.0047	0.9570	−0.0090	0.0095
CGRS(n=4)	0.0044	0.0024	0.0720	−0.0004	0.0091
WGRS(n=4)	0.0027	0.0021	0.2040	−0.0015	0.0068
CGRS(n=6)	0.0046	0.0024	0.0550	−0.0001	0.0094
WGRS(n=6)	0.0033	0.0017	0.0520	0.0000	0.0066
DBP					
CGRS(n=2)	−0.0010	0.0012	0.4040	−0.0033	0.0013
WGRS(n=2)	−0.0018	0.0022	0.4010	−0.0062	0.0025
CGRS(n=4)	−0.0005	0.0011	0.6550	−0.0026	0.0016
WGRS(n=4)	−0.0001	0.0010	0.9580	−0.0019	0.0018
CGRS(n=6)	−0.0004	0.0011	0.7170	−0.0025	0.0017
WGRS(n=6)	0.0000	0.0008	0.9550	−0.0015	0.0015

BMI, body mass index; CGRS, count genetic risk score; LCI, lower confidence interval; SD, standard deviation; WGRS, weighted genetic risk score; UCI, upper confidence interval

Ⅲ. Gene-environment interaction study

1. Study Aim and Hypotheses

1.1. Study Aim

Our first aim was to analyze the effects of the SNP-obesity interactions on hypertension using longitudinal data from the Korean Genome and Epidemiology Study. Because previous studies failed to account for biological mechanisms, we chose genes that have a plausible relationship between obesity and hypertension: insulin resistance, vascular alterations, and RAAS. The second aim of this study was to compare the contribution of the SNPs themselves and the interactions between SNPs or GRS and obesity to the development of hypertension.

1.2. Hypotheses

Hypothesis 1: There are SNPs that have significant gene-environment interactions for incident hypertension.

Hypothesis 2: The contribution of the interaction between the SNPs or GRS and obesity was more than that of the SNPs themselves to the

development of hypertension.

2. Materials and methods

2.1. Study population

We conducted a prospective follow-up investigation using data from the Korean Genome and Epidemiology Study (KoGES).

2.2. Genotypes

We selected 76 genes related to insulin resistance, vascular alterations, and RAAS as shown in a previous study; the gene list is shown in Table 10. [46-48]

Overall, we selected 3608 SNPs from 76 genes.

Table 10. Selected genes related to insulin resistance, vascular alterations and RAAS.

Insulin resistance	Vascular alterations	RAS
ADAMTS9	APOB	REN
CAPN10	APOE	AGT
TCF7L2	ABCG5	ACE
INSR	ABCG8	AGTR1
HMGA1	PCSK9	CYP11B2
ENPP1	SORT1	CYP17A1
PTPN1	ABO	MRAS
IRS1	LDLR	
IRS2	LPA	
AHSG	ANKS1A	
PREX1	TRIB1	
LIN28A	APOA5	
LIN28B	LRP1	
SLC2A4	CETP	
FOXO1	CRP	
PPARG	GATA2	
HNF4	ITGA2	
SREBF1	ITGB3	

FTO	GP6
PPARGC1A	F5
ADIPOQ	F2
ADIPOR1	F7
ADIPOR2	SERPINE1
RETN	ABCA1
IGF1	MTHFR
IGF2BP2	HMOX1
SGK1	CX3CR1
SHBG	LPL
LEPR	IL6
G6PC2	EDN1
GCKR	EDN2
TRIB3	EDN3
	EDNRA
	EDNRB
	NOS1
	NOS2
	VCAM1

Abbreviations: RAAS, renin angiotensin aldosterone system

2.3. Obesity and covariates

Three obesity variables were derived from the KoGES: BMI, waist circumference (WC), and waist-to-hip ratio (WHR). The BMI was calculated as weight in kilograms divided by height in meters squared (kg/m^2) at the baseline survey. WC (cm) was measured three times at the narrowest point between the lower rib and the iliac crest with the participant standing. Similarly, hip circumference (cm) was measured three times at the widest part over the greater trochanters. Means of the three measurements of WC and hip circumference were used. WHR was calculated by dividing WC by hip circumference at the baseline survey. Obesity was defined for each of the three measurements: BMI cut-off $\geq$25 kg/m^2, WC cut-off $\geq$90 cm for males and $\geq$85 cm for females, and WHR cut-off $\geq$0.9 for males and $\geq$0.85 for females at the baseline survey.

We used age (years), sex (male, female), area (Ansung, Ansan), and education ($\leq$9, >9 years of school) at the baseline survey.

2.4. Hypertension assessment

BP was measured using mercury sphygmomanometers (Baumanometer; WA Baum, Copiague, NY, USA) according to a standardized protocol. [42] All measurements in the present study were taken after at least a 5-min rest. We used an average of three measurements. At baseline, hypertensive participants were defined as having SBP ≥140 mmHg or DBP ≥90 mmHg, using antihypertensive medication, or having a history of hypertension diagnosed by a doctor. After these participants were excluded, newly diagnosed cases of hypertension were defined as SBP ≥140 or DBP ≥90 mmHg and taking antihypertensive drugs during the 10-year follow-up. We used incident hypertension data that excluded baseline hypertension participants.

2.5. Statistical analysis

We performed Cox's proportional hazard model for the new development of hypertension:

$$y = \beta_0 + \beta_{cov} \times X_{cov} + \beta_1 \times \text{obesity} + \beta_2 \times \text{SNP} + \beta_3 \times \text{SNP} \times \text{obesity} + e.$$

where X_{cov} refers to the co-variables of age (years), area (Ansung, Ansan), sex (male, female), and education (≤ 9, >9 years of school).

We conducted one degree-of-freedom (1df) analysis of the SNP's main effect, obesity's main effect, and the SNP-obesity interaction using the maximum likelihood estimators of the parameters. Next, we evaluated the joint two degrees-of-freedom (2df) analysis of the SNP's main effect and the SNP-obesity interaction. Randall et al. [38] argued that the 1df test was useful for informing public health interventions by which the environment may attenuate or exacerbate genetic predisposition to disease. Cornelis et al.[49], on the other hand, argued that the 2df test is often much more powerful than the 1df test when the investigators are interested in discovering new markers leveraging potential gene-environmental interactions. Although there is no agreement upon the significance threshold for

interaction studies [50], previous studies used Bonferroni-corrected significant joint 2df test in conjunction with a nominally significant ($p < 0.05$) 1df interaction test. [29] Because we examined 3608 selected SNPs, we used a Bonferroni-corrected 2df joint P value of 2.0×10^{-6} (~ 0.01/3608) as the criteria in addition to a 1df P value < 1.0×10^{-2} for significance level.

The GRS was calculated for those SNPs showing significant interaction with obesity for hypertension incidence [$P < 2 \times 10^{-6}$ (2df test) and $P < 1 \times 10^{-2}$ (1df test)]. To search for any linkage between the significant SNPs, we calculated $|D'|$ values and drew linkage disequilibrium plots. The GRS was produced by two methods: a simple count method (count GRS) and a weighted method (weighted GRS) [44, 45]. We assumed an additive genetic model for each SNP, applying a linear weighting of 0, 1, or 2 to genotypes containing 0, 1, or 2 risk alleles, respectively. The simple count model assumes that each SNP in the panel contributes equally to the risk of hypertension incidence and was calculated by summing the values for each of the SNPs. The weighted GRS was calculated by multiplying each β coefficient by the number of corresponding risk alleles (0, 1, and 2).

If the β coefficient was negative, we assumed that the major allele was the risk allele. All β coefficients were positive because the coded allele was always the risk allele. The GRS was categorized by median (median of count GRS = 2; median of weighted GRS = 0.08). The hypertension incidence risk associated with the genotype was estimated together with the 95% confidence interval (CI) and computed using Cox' s proportional hazard model with an additive genetic model. The percentage of variance explained by each obesity measurement and GRS for hypertension was estimated using generalized linear modeling.

All statistical analyses were performed using SAS software version 9.3 (SAS Institute Inc., Cary, NC, USA), Plink (version 1.08, http://pngu.mgh.harvard.edu/~purcell/plink), and R version 3.1.0 (Comprehensive R Archive Network: http://cran.r-project.org). Haploview 4.2 (www.broadinstitute.org/haploview) was used for the linkage disequilibrium plots.

3. Results

3.1. General characteristics

Among the 8832 participants, 4179 (47.3%) were men. The average age was 52 (SD 8.92) years, and the average BMI was 24.6 (SD 3.12) kg/m^2. At baseline, hypertension was diagnosed in 2971 (33.6%) participants, and the remaining 5861 (66.4%) participants were not hypertensive. During the 10-year follow-up, hypertension was newly detected in 1409 participants (first follow-up: 436; second follow-up: 274; third follow-up: 232; fourth follow-up: 322; and fifth follow-up: 145). As shown in Table 11, there were statistically significant differences in age, sex, area, education, BMI, WC, and WHR measured between the new hypertension and non-hypertensive groups.

Table 11. General characteristics of the study participants diagnosed with hypertension or non-hypertensives.

Variable		No hypertension	New hypertension	*P-value*
Total no. (n=5379)		4452 (78.1)	1409 (21.9)	
Area	Ansung	1625 (36.5)	799 (56.7)	<0.001
	Ansan	2827(63.5)	610 (43.3)	
Age (years)		49.2 (8.0)	53.5 (8.8)	<0.001
Sex	Male	2061 (46.3)	714 (50.7)	0.0041
	Female	2391 (53.7)	695 (49.3)	
Education (years of	≤9	2077 (46.9)	868 (62.2)	<0.001

school)				
	>9	2350(53.8)	527 (37.8)	
	Missing	39		
BMI (kg/m^2)	<25	2951 (66.3)	760(54.0)	<0.001
	≥25	1500 (33.7)	648 (46.0)	
	Missing	2		
WC (cm)	Male: <90, female: <85	3603 (81.0)	931 (66.1)	<0.001
	Male: ≥90, female: ≥85	846(19.0)	478 (33.9)	

	Missing	3		
WHR	Male: <0.9, female: <0.85	2719(61.1)	565(40.0)	<0.001
	Male: ≥0.9, female: ≥0.85	1730 (38.9)	843 (60.0)	
	Missing	4		

*χ^2 test and Student's *t*-test were used for categorical and continuous variables, respectively.

BMI, body mass index; WC, waist circumference; WHR, waist: hip ratio.

3.2 Gene-environment interaction

Four loci reaching statistical significance at P-value$<2 \times 10^{-6}$ (2df test) and P-value$<1 \times 10^{-2}$ (1df test) were related to the development of hypertension using the follow-up data (Table 12). The interactions between WHR and rs6020611 and rs754118 on *PTPN1* (protein tyrosine phosphatase, non-receptor type 1) were associated with the incidence of hypertension. The interactions between WC and rs3817588 on *GCKR* (glucokinase (hexokinase 4) regulator) and rs1864815 on *ABCG5* (ATP-binding cassette, subfamily G (WHITE), member 5) were also associated with the development of hypertension.

Table 12. Significant SNP-obesity interactions for those newly diagnosed with hypertension using Cox's proportional hazard model

Obesity	Chr.	Position	SNP	minor	major	MAF	GEN	SNP	main beta	main SE	2df int.	1df int.
BMI	1	230714140	rs5050	G	T	0.141	AGT	Imp	0.100	0.060	3.71E-10	0.0479
BMI	2	21002613	rs1801702	G	C	0.020	APOB	Imp	-0.081	0.147	1.08E-06	0.0375
BMI	4	23933430	rs4697428	C	T	0.181	PPARGC1A	Imp	0.003	0.055	6.08E-08	0.0249
BMI	17	27778906	rs1137933	A	G	0.104	NOS2	Imp	0.074	0.066	4.03E-07	0.0359
BMI	17	17821475	rs11656665	A	G	0.063	SREBF1	Imp	0.093	0.086	5.05E-07	0.0267
WC	1	230712956	rs2004776	C	T	0.399	AGT	Imp	0.034	0.044	1.83E-06	0.0328
WC	**2**	**43816313**	**rs1864815**	**T**	**A**	**0.112**	**ABCG5**	**Imp**	**0.116**	**0.066**	**2.55E-07**	**0.0079**

WC	**2**	**27508345**	**rs3817588**	**C**	**T**	**0.338**	**GCKR**	**Imp**	**−0.040**	**0.046**	**3.46E−07**	**0.003**
WC	2	43816254	rs4953019	A	G	0.112	ABCG5	Imp	0.118	0.066	7.90E−06	0.0068
WC	3	185794573	rs16860235	A	G	0.011	IGF2BP2	Imp	−0.257	0.231	7.63E−07	0.0325
WC	17	27756664	rs7406657	C	G	0.361	NOS2	Imp	0.060	0.044	1.78E−06	0.0458
WC	20	48800635	rs7360629	A	G	0.011	PREX1	Imp	0.137	0.194	2.74E−07	0.0464
WHR	2	43816313	rs1864815	T	A	0.112	ABCG5	Imp	0.116	0.066	4.74E−07	0.0399
WHR	3	64554976	rs13059202	G	A	0.378	ADAMTS9	Imp	0.080	0.044	7.85E−07	0.0249
WHR	3	64627768	rs6445419	G	C	0.473	ADAMTS9	Imp	−0.007	0.044	6.98E−12	0.0492
WHR	4	24038932	rs10025406	T	C	0.312	PPARGC1A	Imp	0.119	0.046	5.05E−06	0.0195
WHR	12	117389837	rs7299612	T	C	0.122	NOS1	Imp	0.096	0.063	2.38E−07	0.0341
WHR	16	54086472	rs11076017	C	T	0.435	FTO	Imp	−0.069	0.045	1.19E−08	0.0496

WHR	16	54007341	rs1971037	T	C	0.490	FTO	Imp	0.013	0.044	4.72E-09	0.0243
WHR	17	27778906	rs1137933	A	G	0.104	NOS2	Imp	0.074	0.066	3.27E-06	0.003
WHR	20	50563528	rs2145697	T	C	0.095	PTPN1	Imp	0.012	0.076	2.08E-08	0.0419
WHR	20	50579630	rs2282147	T	C	0.281	PTPN1	Imp	0.002	0.049	5.82E-06	0.0068
WHR	20	50581790	rs2426164	G	A	0.281	PTPN1	Imp	0.002	0.049	5.82E-06	0.0068
WHR	20	50578956	rs4809800	C	A	0.281	PTPN1	Imp	0.002	0.049	5.82E-06	0.0068
WHR	20	50511703	rs6020572	A	G	0.297	PTPN1	Imp	0.014	0.048	2.51E-07	0.0414
WHR	20	50573995	rs6020608	T	C	0.093	PTPN1	Imp	0.003	0.077	5.10E-09	0.0368
WHR	**20**	**50578070**	**rs6020611**	**A**	**G**	**0.281**	**PTPN1**	**Imp**	**0.002**	**0.049**	**1.15E-06**	**0.0069**
WHR	20	50536246	rs6067484	G	A	0.096	PTPN1	Imp	0.027	0.075	8.42E-08	0.0376
WHR	**20**	**50575367**	**rs754118**	**T**	**C**	**0.280**	**PTPN1**	**Imp**	**0.004**	**0.049**	**6.05E-07**	**0.0084**

WHR	20	50580666	rs914460	C	T	0.281	PTPN1	Imp	0.002	0.049	5.82E−06	0.0068

Significant SNPs are displayed in bold. (P−value<2×10^{-6} (2df test) and P−value<1×10^{-2} (1df test))

Df, degree of freedom; int., interaction effect; Imp, imputed; MAF, minor allele frequency;

main, main effect; SE, standard error; SNP, single nucleotide polymorphism; WC, waist circumference;

WHR, waist: hip ratio.

Among the significant SNPs related to hypertension development, SNPs on two loci (rs6020611 and rs754118) on *PTPN1* were found to be in high linkage disequilibrium ($|D'| \geq 0.9$; see Figure 9). Therefore, we selected one representative SNP from the closely linked SNPs based on the estimated size of the main genetic analysis results. Thus, rs754118 on *PTPN1* was selected for GRS, assuming that the estimated effect in the selected SNPs could represent the other closely linked SNPs. We used rs1864815 from *ABCG5*, rs754118 from *PTPN1*, and rs3817588 from *GCKR* in applying GRS to evaluate the combined effects of the three significant risk alleles. We calculated the risk scores using a simple allele count (count GRS) or a weighted approach (weighted GRS), and GRS was divided into two groups (less than the median and greater or more than the median). Four combinations were used to evaluate the interaction between obesity and GRS for hypertension incidence: (1) low GRS and no obesity (reference group), (2) high GRS and no obesity, (3) low GRS and obesity, and (4) high GRS and obesity. The HR (95% CI) according to these four combinations was shown, and there is a

different HR according to the GRS-obesity combination (Figure 10).

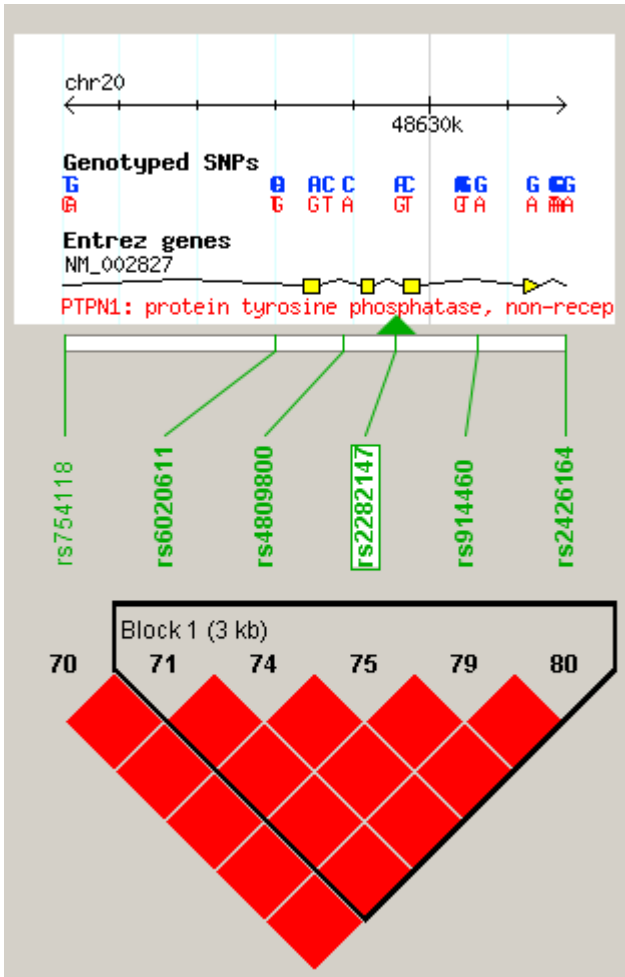

Figure 9. Linkage disequilibrium plots for the selected PTPN1 SNPs

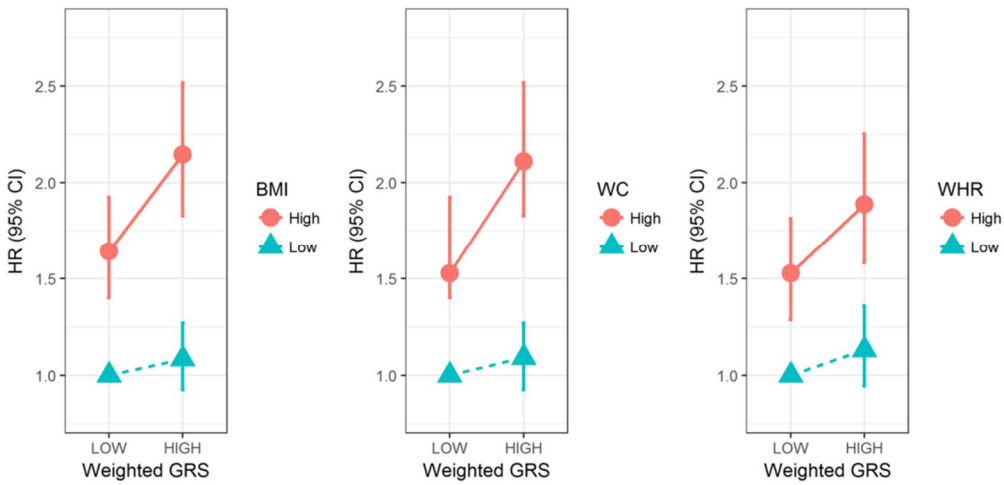

Figure 10. HR of hypertension compared with reference group (low GRS and low BMI, WC, and WHR).

BMI, body mass index; CI, confidence interval; GRS, genetic risk score; HR, hazard ratio; WC, waist circumference; WGRS, weighted genetic risk score; WHR, waist: hip ratio.

3.3. Sensitivity analysis

We also conducted sensitivity analysis with further adjustment for baseline systolic blood pressure levels (Figure 11). The analysis results did not differ from the original results.

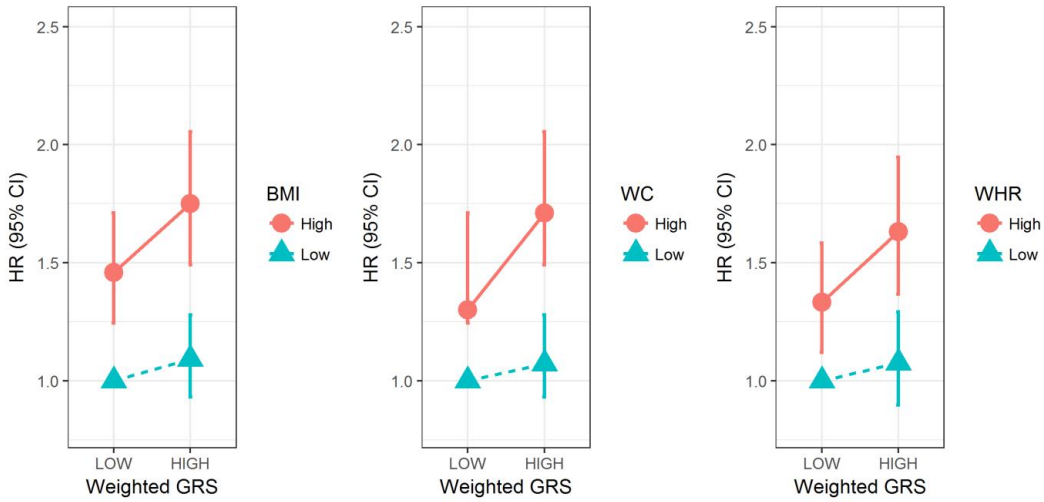

Figure 11. HR of hypertension compared with reference group (low GRS and low BMI, WC, and WHR).

Covariates: area, age, sex, education, baseline systolic blood pressure.

3.4. Contributory proportions

The contributory proportions of BMI, WC, and WHR that explained hypertension development were 2.05%, 6.46%, and 7.62%, respectively. We also found that the proportions of count GRS and weighted GRS that explained hypertension development were 0.04% and 0.09%, respectively. The increment in the contributory proportions of BMI, WC, and WHR that explained hypertension, from the low to the high weighted GRS, was from 2.10% to 3.00% (0.90%), from 5.26% to 9.08% (3.82%), and from 6.60% to 9.25% (2.65%), respectively.

Ⅳ. Discussion

1. Mendelian Randomization study

Using the data from a 10-year follow-up investigation including 8832 community-dwelling Korean middle-aged adults, we performed an analysis utilizing a MR design and provided additional evidence to support the causal role of BMI in hypertension. These findings are consistent with evidence from observational studies that have demonstrated the association of high BMI with an increased risk of hypertension. [13] This evidence provides a rationale to further investigate whether weight-control programs can reduce the incidence of hypertension in those who are at risk.

Several pathogenic mechanisms have been suggested to contribute to the development of hypertension in an obese population: insulin resistance, vascular alterations, and activation of the renin-angiotensin-aldosterone system. [31, 32] Excess adipocyte tissue stimulates insulin secretion, which activates the sympathetic nervous system and raises the BP. [33] Insulin also acts directly on the kidneys to stimulate sodium retention, increase plasma volume, and

raise the BP. [34] Vascular alterations, including structural changes, endothelial dysfunction, and altered stiffness are common in obesity and are also thought to contribute to the development of hypertension. [35, 36] An activated renin–angiotensin–aldosterone system in the presence of the excess adipose tissue of obese people generates angiotensin and aldosterone, which again elevate the BP. [31]

An important difference between conventional RCTs and MR studies using genetic polymorphisms is that MR studies evaluate the association between lifetime exposure to selected alleles in the general population with an outcome, whereas conventional RCTs provide insights for shorter periods among more selected individuals. [51]

Previously, a small number of MR studies have provided evidence supporting a causal link between BMI and hypertension. Fall T et al. demonstrated a significant association between the adiposity–associated variant rs9939609 at the FTO locus and systolic blood pressure and suggested a possible causal association with elevated systolic blood pressure (+0.89 mmHg/(kg/m^2)). [20] In this study, rs9939609 at the FTO locus was included in the genetic risk score.

Fall T et al. also constructed a GRS using 32 SNPs and reported a causal effect of adiposity on blood pressure within the European Network for Genetic and Genomic Epidemiology Consortium.[21] Holmes et al. performed a genetic-association study of BMI using the CardioChip, then used the results to construct a GRS comprising 14 SNPs that showed that a 1 kg/m^2 increase in BMI increased SBP by 0.70 mmHg (95% CI: 0.24-1.16) and DBP by 0.28 mmHg (95% CI: 0.03-0.52) in the US population. [22] One recent study also showed a causal relationship between WHR and blood pressure. [52] (Table 13)

We conducted a meta-analysis between BMI and a binary measure of hypertension. (Figure 12) From the pooled analysis, we identified an odds ratio (OR) of 1.14 for risk of hypertension per 1 kg/m^2 increase of BMI (95% confidence interval (CI): 1.08- 1.20, test for heterogeneity between studies I^2=40.6%, P_{het}=0.194). We also conducted a meta-analysis of an MR study between BMI and a continuous measure of blood pressure. (Figure 13) The beta-estimate for SBP was 0.25 (95% CI: 0.02- 0.48, test for heterogeneity between studies I^2=86.7%, P_{het}<0.001). The beta-

estimate for DBP was 0.18 (95% CI: 0.01–0.36, test for heterogeneity between studies I^2=86.2%, P_{het}<0.001).

Table 13. Previous Mendelian randomization studies of obesity and hypertension

Author, year, country (enrollment period)	Ref.	Number	exposure	Outcomes	Note
Emdin, 2017, UK (2007–2015)	[52]	111986	WHR	2.1 mm Hg [95% CI, 1.2–3.0] higher SBP per 1 SD increase in WHR	UK biobank
Fall, 2015, Europe	[21]	66,997	BMI	DBP 0.15 (0.03–0.26), SBP 0.16 (0.04–0.28) per 1 kg/m^2 increase in BMI	the European Network for Genetic and Genomic Epidemiology (ENGAGE) Consortium
Holmes, 2014,	[22]	30,136	BMI	SBP (0.70 mmHg; 95% CI = 0.24–1.16),	

USA, Europe, Australia (1987–2006)				DBP 0.28 (0.03–0.52) per 1 kg/m^2 increase in BMI		
Fall, 2013, Europe	[20]	147,644	BMI	DBP 0.490 (0.187,0.793), SBP 0.892(0.475, 1.309) per 1 kg/m^2 increase in BMI	the European Network for Genetic and Genomic Epidemiology (ENGAGE) Consortium	

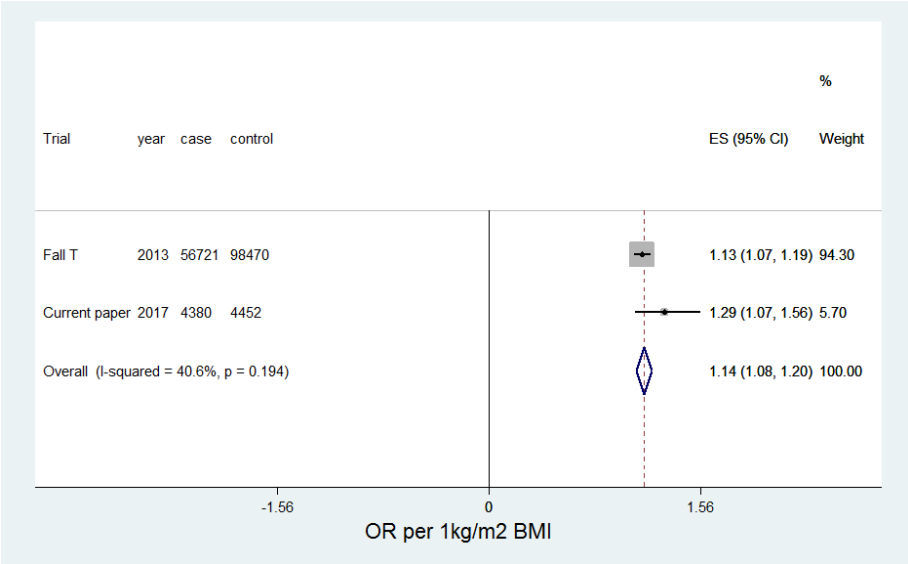

Figure 12. Forest plot of association analyses between BMI and hypertension

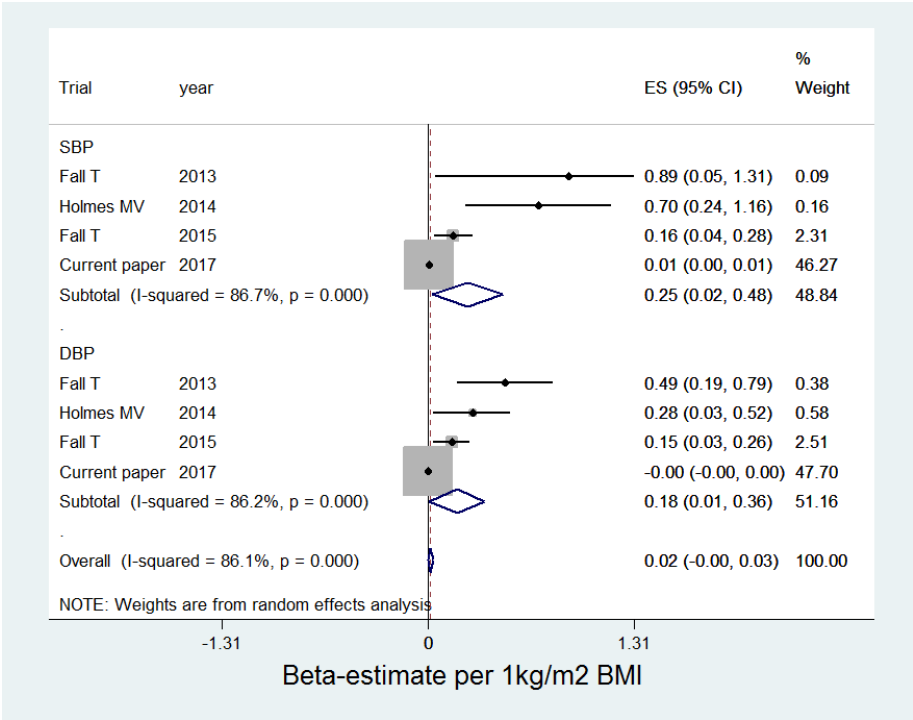

Figure 13. Forest plot of association analyses between BMI and blood pressure

An MR study is a valid way to explore evidence for causality, given that certain assumptions are met. First, there has to be a strong association between a genetic variant (IV) and the exposure of interest. Two SNPs (rs17178527 and rs9939609) used in this study have previously been shown to be strongly associated with BMI [40, 53, 54], a finding that was replicated in our present study. To assess the relevance of the instruments, we tested the F-statistic in the first-stage regression (IV association with the risk exposure). As a rule of thumb, if the F-statistic was smaller than 10, the IV was defined as a "weak instrument". [55] In our study, the F statistics for all BMI GRSs were greater than 10 (52.7-125.3), so problems associated with weak instruments were unlikely. Second, the IV must be independent of covariates. In our study, the IV was independent with measurable covariates (age, sex, area, education, smoking, and alcohol consumption). Third, there are no other pathways between the genetic variant and the outcome (pleiotropy). However, this assumption is untestable. The rs9939609 SNP on the FTO gene has no known pleiotropy.[20] However, the other SNPs were not validated to exclude pleiotropy. Because the quality of evidence

provided by a Mendelian randomization study relies heavily on these assumptions[56], and these MR analyses using six different GRSs provided consistent results, although GRS (n=2) yielded a greater OR than did GRS (n=4) and GRS (n=6), this difference might be due to the inclusion of additional marginally significant SNPs, which would reduce the strength and precision of a SNP-exposure association. Likewise, Vassy et al. found that a 62-SNP GRS did not substantively improve the prediction of type 2 diabetes compared with a 40-SNP GRS.[57] More work is needed to determine whether SNPs that do not reach stringent genome-wide significance levels in GRSs should be included in MR studies.

Our main MR analysis considered both prevalent and incident hypertension cases. Additional sensitivity analyses (except WGRS (n=6)) using only prevalent cases at baseline also showed a causal effect of adiposity on hypertension. In contrast, when we conducted a sensitivity analysis using only an incident case, a causal relationship was not found. Because the Mendel study requires a large sample size,[58] no significant results were obtained when the incident case was analyzed alone.

2. Gene-environment interaction

We identified 4 significant SNPs the interaction with obesity that contributed to hypertension development during the follow-up survey.

The significantly associated locus that interacted with WHR was located on *PTPN1* of chromosome 20, and the locus interacting with WC for incident hypertension was located on *GCKR* of chromosome 2. *PTPN1* codes for the protein tyrosine phosphatase 1B (PTP1B), which is involved in an activated insulin receptor [48]. *GCKR* regulates glucokinase (hexokinase 4) through glucose-stimulated insulin release as a physiological glucose sensor [59]. Therefore, the mechanism by which these genes modulate the relationship between obesity and hypertension could be via insulin resistance. Excess adipocytes in obesity accelerate insulin resistance and hyperinsulinemia, stimulating SNS activity, and finally inducing hypertension[31]. There are some studies examining the interrelationship of *PTPN1* [60-62], *GCKR* [63, 64], and hypertension or hypertension-related disease.

WC significantly interacted with rs1864815 on *ABCG5* on chromosome 2 for hypertension development, and *ABCG5* was

associated with vascular alterations. Genetic polymorphisms of *ABCG5/ABCG8* induce atherosclerosis, which accelerates intestinal absorption of dietary cholesterol and limits biliary excretion of neutral sterols [65]. Therefore, the mechanism by which the *ABCG5* gene affects obesity-related hypertension would be through atherosclerosis. Obese people have more visceral adipose tissue and adipose tissue-resident macrophages, which produce more pro-inflammatory cytokines, such as tumor necrosis factor α and interleukin-6, but less adiponectin. These cytokine changes play a major role in the pathogenesis of endothelial dysfunction, atherosclerosis, and the subsequent hypertension. [32] Several studies have shown that *ABCG5* is related to hypertension. [66, 67]

In our results, WC and WHR interacted with different genes (WHR and *PTPN1*, WC and *GCKR*), but these genes were involved in the same pathogenic mechanism (insulin resistance). In previous epidemiologic studies, WC was a better anthropometric measure to use for identifying individuals with cardiovascular disease risk than WHR [68-71]. WC showed a higher association with insulin resistance than WHR because WC is more closely correlated with the

level of abdominal visceral adipose tissue than WHR. [72] In another Genome-Wide Association Study (GWAS), WC and WHR were also associated with different genes. [73] First, WHR is a ratio indicator and was biologically different from WC. While the waist circumference reflected visceral organs and abdominal (both subcutaneous and intra-abdominal) fat, the hip circumference may represent muscle mass, gluteal fat mass and skeletal frame. WHR is a combination of two circumferences [74], and a change of body fat distribution may produce little or no change in the ratios. [75] In fact, both lean and massively obese individuals may have different WCs but have the same WHR [68]. In abdominal imaging studies, WHR is a poor indicator of changes in visceral fat [76], while WC had excellent correlation with abdominal visceral adipose tissue accumulation [69]. Second, we conducted interaction analysis using a binary variable according to the definition of obesity. Cohen's Kappa coefficient between obesity variables using WC and WHR was 0.48, the 95% confidence interval was (0.46, 0.50), and the amount of agreement was moderate.

In addition to the incident hypertension study, we performed logistic

regression analysis using baseline data but did not find SNPs that were significantly interactive with obesity at 2df $P < 2 \times 10^{-6}$ and 1df $P < 1 \times 10^{-2}$ related to the prevalence of hypertension.

We additionally analyzed gene changes in the abdominal obesity interaction on the development of hypertension. The definition of change in abdominal obesity (WC and WHR) was the difference between baseline and the last follow-up examination. We found 3 significant SNPs that interact with change in abdominal obesity for the development of hypertension: the interactions between the change in WC and rs1384872 on *NOS1* (nitric oxide synthase 1) and between the change in WHR and rs2472508 and rs2487049 on *ABCA1* (ATP-binding cassette protein A1) (1df $P < 0.01$, 2df $P < 2 \times 10^{-6}$) (Table 14). These genes were different from the ones we found in the gene-obesity interaction analysis. While *PTPN1* and *GCKR* genes are more related to insulin resistance, these genes were more related to vascular alterations: the *NOS1* gene was involved in endothelial dysfunction [77], and the *ABCA1* gene was involved in cholesterol metabolism. [78] Obesity and change in abdominal obesity may interact with different genes through different mechanisms. It

requires further study to evaluate the specific interactions for the different obesity indicators.

Table 14. Significant SNP−the change in abdominal obesity interactions for individuals with newly diagnosed hypertension using Cox' s proportional hazard model.

Chr.	SNP	Known gene	Type	Minor	Major	MAF	b (main)	SE (main)	*P* (1df int.)	*P* (2df int.)
WC										
12	rs1384872	*NOS1*	Imp	T	C	0.38	0.051	0.045	0.0005	5.37×10^{-8}
WHR										
9	rs2472508	*ABCA1*	Imp	A	G	0.17	−0.05	0.058	0.0028	1.43×10^{-7}
9	rs2487049	*ABCA1*	Imp	G	A	0.168	−0.052	0.058	0.0031	1.22×10^{-8}

Significant SNPs if P $<2x10^{-6}$ (2df test) and P $<1x10^{-2}$ (1df test)

Chr, chromosome; Df, degree of freedom; int., interaction effect; Imp, imputed; MAF, minor allele frequency; main, main effect; SE, standard error; SNP, single nucleotide polymorphism; WC, waist circumference; WHR, waist: hip ratio.

Several GWAS had identified genetic variants in relation to systolic blood pressure (SBP) and diastolic blood pressure (DBP). A recent study reported SBP and DBP heritability at 36% and 27%, respectively [79], but genetic variants explained <3% of the total phenotypic variability. [27] This low genetic contribution was called "missing heritability". [80] When we calculated the accountability by variance with regard to the relation between obesity indices (BMI, WC, and WHR) and genetic score (count GRS and weighted GRS), we found that direct genetic contribution was relatively small, whereas the contribution by obesity was bigger than that for the genetic factor: 2.05–7.62% for obesity and 0.04–0.09% for the genetic factor. This result, shown in Figure 10, indicates that the HR of hypertension development in the obesity group was significantly higher than that in the non-obesity group, and, in addition, high GRS elevated the HR more compared with low GRS. This finding suggested that genetic variants increase the effect of obesity further, although they have relatively smaller effects than obesity on hypertension.

3. Strengths and limitations

The strengths of the present study are the well-defined community setting and a relatively large sample. To our knowledge, this is the first report showing the effect of common genetic variations related to BMI as the IV in measuring the association with hypertension in an East Asian population. It is also the first report using longitudinal data of a gene-obesity interaction affecting incident hypertension. We also used pathway-related genes that are biologically plausible to explain the gene-obesity interaction and analyzed the GRS score to show the combined effects of genetic variants on obesity.

With regard to the limitations of the present study, first, we built the BMI GRS based only on common variants, so we were unable to assess the potential contribution of rare variants. Second, the results may not be generalizable to populations of different ethnicities because we used a cohort composed only of Koreans. Third, this study examined the causal effect of obesity on BP, but we could not test the impact of acute changes. Finally, there was no question relating to the length of time of use of the hypertensive drug. Therefore, the occurrence of hypertension was measured only at the

time of follow-up, which was every two years.

In the gene-environment study, with regard to the limitations, the validity of our findings was somewhat limited because we used only a single study. Further validation and replication in other independent data, particularly of a community cohort with genetic information, would be necessary. Second, the result of our study was limited in its application to populations of different ethnicities, because we used a cohort study conducted in Koreans only. Third, we used prospective, community-based cohort data with a follow-up rate of 62.1% for 10 years. Even though this follow-up rate is not considerably low, there is a possibility of loss to follow-up bias. Finally, because most of our results were imputed SNPs, our study was likely to be underpowered. Therefore, combining data across multiple studies will be necessary to detect any gene-environment interactions.

Ⅴ. Conclusion

We found that the genetic predisposition for a higher BMI was associated with a higher risk of hypertension in the Korean population. This MR analysis provided evidence of a causal relationship between BMI and hypertension. Our results suggest that controlling obesity may be beneficial for the prevention of hypertension.

We also identified 4 significant genetic variants affecting incident hypertension in the Korean population by interaction effects using longitudinal data. In addition, we observed that the increment in the contributory proportions of obesity that explained the development of hypertension by the change in genetic risk scores was greater than the contributory proportions of the genetic risk scores themselves. Therefore, obese individuals with susceptible genes for the development of hypertension will require more blood pressure control.

References

1. Lim, S.S., et al., *A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010.* Lancet, 2012. **380**(9859): p. 2224-60.
2. Zhou, B., et al., *Worldwide trends in blood pressure from 1975 to 2015: a pooled analysis of 1479 population-based measurement studies with 19·1 million participants.* The Lancet. **389**(10064): p. 37-55.
3. Ng, M., et al., *Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013.* The Lancet. **384**(9945): p. 766-781.
4. 질병관리본부, *2015 건강행태 및 만성질환 통계*. 2016: Korea.
5. Lee, J.W., et al., *Trends in socioeconomic costs of morbid obesity among Korean adults, 2009-2013: Data from National Health Insurance Service.* Obes Res Clin Pract, 2017.
6. Ezzati, M. and E. Riboli, *Behavioral and dietary risk factors for noncommunicable diseases.* N Engl J Med, 2013. **369**(10): p. 954-64.
7. Stokes, J., 3rd, et al., *Blood pressure as a risk factor for cardiovascular disease. The Framingham Study--30 years of follow-up.* Hypertension, 1989. **13**(5 Suppl): p. I13-8.
8. Kannel, W.B., *Fifty years of Framingham Study contributions to*

understanding hypertension. J Hum Hypertens, 2000. **14**(2): p. 83-90.

9. Hall, J.E., et al., *OBESITY-INDUCED HYPERTENSION: INTERACTION OF NEUROHUMORAL AND RENAL MECHANISMS*. Circulation research, 2015. **116**(6): p. 991-1006.

10. Susic, D. and J. Varagic, *Obesity: A Perspective from Hypertension.* Med Clin North Am, 2017. **101**(1): p. 139-157.

11. Moore, L.L., et al., *Weight loss in overweight adults and the long-term risk of hypertension: the Framingham study.* Arch Intern Med, 2005. **165**(11): p. 1298-303.

12. Chandra, A., et al., *The relationship of body mass and fat distribution with incident hypertension: observations from the Dallas Heart Study.* J Am Coll Cardiol, 2014. **64**(10): p. 997-1002.

13. Lee, S.K., et al., *Obesity phenotype and incident hypertension: a prospective community-based cohort study.* J Hypertens, 2013. **31**(1): p. 145-51.

14. Lee, J.W., et al., *Anthropometric indices as predictors of hypertension among men and women aged 40-69 years in the Korean population: the Korean Genome and Epidemiology Study.* BMC Public Health, 2015. **15**: p. 140.

15. Smith GD, E.S.M.R.G.V.a.I.f.S.C.I.i.O.S.I.N.R.C.U.C.o.A.i.C.a., *Biosocial Surveys*. 2008: Washington (DC): National Academies Press (US).

16. Smith, G.D. and S. Ebrahim, *Mendelian randomization: prospects, potentials,*

and limitations. International Journal of Epidemiology, 2004. **33**(1): p. 30-42.

17. Horvath, K., et al., *Long-term effects of weight-reducing interventions in hypertensive patients: systematic review and meta-analysis.* Arch Intern Med, 2008. **168**(6): p. 571-80.

18. Tyson, C.C., et al., *Impact of 5-year weight change on blood pressure: results from the Weight Loss Maintenance trial.* J Clin Hypertens (Greenwich), 2013. **15**(7): p. 458-64.

19. Madsbad, S., C. Dirksen, and J.J. Holst, *Mechanisms of changes in glucose metabolism and bodyweight after bariatric surgery.* Lancet Diabetes Endocrinol, 2014. **2**(2): p. 152-64.

20. Fall, T., et al., *The role of adiposity in cardiometabolic traits: a Mendelian randomization analysis.* PLoS Med, 2013. **10**(6): p. e1001474.

21. Fall, T., et al., *Age- and sex-specific causal effects of adiposity on cardiovascular risk factors.* Diabetes, 2015. **64**(5): p. 1841-52.

22. Holmes, M.V., et al., *Causal effects of body mass index on cardiometabolic traits and events: a Mendelian randomization analysis.* Am J Hum Genet, 2014. **94**(2): p. 198-208.

23. WHO, *Global status report on noncommunicable diseases 2014*, in *Attaining the nine global noncommunicable diseases targets; a shared responsibility*. 2014.

24. OECD, *OECD Health Statistics 2014*. 2014.

25. Levy, D., et al., *Framingham Heart Study 100K Project: genome-wide*

associations for blood pressure and arterial stiffness. BMC Med Genet, 2007. **8 Suppl 1**: p. S3.

26. Tarnoki, A.D., et al., *Heritability of central blood pressure and arterial stiffness: a twin study.* J Hypertens, 2012. **30**(8): p. 1564-71.

27. Ehret, G.B., *Genome-wide association studies: contribution of genomics to understanding blood pressure and essential hypertension.* Curr Hypertens Rep, 2010. **12**(1): p. 17-25.

28. Basson, J., et al., *Gene-education interactions identify novel blood pressure loci in the Framingham Heart Study.* Am J Hypertens, 2014. **27**(3): p. 431-44.

29. Simino, J., et al., *Gene-alcohol interactions identify several novel blood pressure loci including a promising locus near SLC16A9.* Front Genet, 2013. **4**: p. 277.

30. Sung, Y.J., et al., *Gene-Smoking Interactions Identify Several Novel Blood Pressure Loci in the Framingham Heart Study.* Am J Hypertens, 2014.

31. Rahmouni, K., *Obesity-associated hypertension: recent progress in deciphering the pathogenesis.* Hypertension, 2014. **64**(2): p. 215-21.

32. Vaneckova, I., et al., *Obesity-related hypertension: possible pathophysiological mechanisms.* J Endocrinol, 2014. **223**(3): p. R63-r78.

33. Landsberg, L., et al., *Obesity-related hypertension: pathogenesis, cardiovascular risk, and treatment: a position paper of The Obesity Society and the American Society of Hypertension.* J Clin Hypertens (Greenwich),

2013. **15**(1): p. 14-33.

34. DeFronzo, R.A., *Insulin and renal sodium handling: clinical implications.* Int J Obes, 1981. **5 suppl 1**: p. 93-104.

35. De Ciuceis, C., et al., *Effects of weight loss on structural and functional alterations of subcutaneous small arteries in obese patients.* Hypertension, 2011. **58**(1): p. 29-36.

36. Weisbrod, R.M., et al., *Arterial stiffening precedes systolic hypertension in diet-induced obesity.* Hypertension, 2013. **62**(6): p. 1105-10.

37. Xi, B., et al., *Influence of obesity on association between genetic variants identified by genome-wide association studies and hypertension risk in Chinese children.* Am J Hypertens, 2013. **26**(8): p. 990-6.

38. Randall, J.C., et al., *Sex-stratified Genome-wide Association Studies Including 270,000 Individuals Show Sexual Dimorphism in Genetic Loci for Anthropometric Traits.* PLoS Genetics, 2013. **9**(6): p. e1003500.

39. Kim, Y.K., et al., *Identification of a genetic variant at 2q12.1 associated with blood pressure in East Asians by genome-wide scan including gene-environment interactions.* BMC Med Genet, 2014. **15**: p. 65.

40. Cho, Y.S., et al., *A large-scale genome-wide association study of Asian populations uncovers genetic factors influencing eight quantitative traits.* Nat Genet, 2009. **41**(5): p. 527-34.

41. KHPF, *Low Alcohol Consumption Guideline*. 2013, KHPF: Seoul, Korea. p. 5.

42. *1999 World Health Organization-International Society of Hypertension*

Guidelines for the Management of Hypertension. Guidelines Subcommittee. J Hypertens, 1999. **17**(2): p. 151-83.

43. Tobin, M.D., et al., *Adjusting for treatment effects in studies of quantitative traits: antihypertensive therapy and systolic blood pressure.* Stat Med, 2005. **24**(19): p. 2911-35.

44. Cornelis, M.C., et al., *Joint effects of common genetic variants on the risk for type 2 diabetes in U.S. men and women of European ancestry.* Ann Intern Med, 2009. **150**(8): p. 541-50.

45. Ripatti, S., et al., *A multilocus genetic risk score for coronary heart disease: case-control and prospective cohort analyses.* Lancet, 2010. **376**(9750): p. 1393-400.

46. Kotsis, V., et al., *Mechanisms of obesity-induced hypertension.* Hypertens Res, 2010. **33**(5): p. 386-93.

47. Montagnana, M., E. Danese, and G. Lippi, *Genetic risk factors of atherothrombosis.* Pol Arch Med Wewn, 2014. **124**(9): p. 474-82.

48. Honardoost, M., et al., *Insulin resistance associated genes and miRNAs.* Appl Biochem Biotechnol, 2014. **174**(1): p. 63-80.

49. Cornelis, M.C., et al., *Gene-environment interactions in genome-wide association studies: a comparative study of tests applied to empirical studies of type 2 diabetes.* Am J Epidemiol, 2012. **175**(3): p. 191-202.

50. Hamza, T.H., et al., *Genome-wide gene-environment study identifies glutamate receptor gene GRIN2A as a Parkinson's disease modifier gene*

via interaction with coffee. PLoS Genet, 2011. **7**(8): p. e1002237.

51. Mokry, L.E., et al., *Vitamin D and Risk of Multiple Sclerosis: A Mendelian Randomization Study.* PLoS Med, 2015. **12**(8): p. e1001866.

52. Emdin, C.A., et al., *Genetic association of waist-to-hip ratio with cardiometabolic traits, type 2 diabetes, and coronary heart disease.* JAMA, 2017. **317**(6): p. 626-634.

53. Frayling, T.M., et al., *A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity.* Science, 2007. **316**(5826): p. 889-94.

54. Willer, C.J., et al., *Six new loci associated with body mass index highlight a neuronal influence on body weight regulation.* Nat Genet, 2009. **41**(1): p. 25-34.

55. Staiger, D. and J.H. Stock, *Instrumental Variables Regression with Weak Instruments.* Econometrica, 1997. **65**(3): p. 557-586.

56. Burgess, S., A.S. Butterworth, and J.R. Thompson, *Beyond Mendelian randomization: how to interpret evidence of shared genetic predictors.* Journal of Clinical Epidemiology, 2016. **69**: p. 208-216.

57. Vassy, J.L., et al., *Polygenic type 2 diabetes prediction at the limit of common variant detection.* Diabetes, 2014. **63**(6): p. 2172-82.

58. Burgess, S., *Sample size and power calculations in Mendelian randomization with a single instrumental variable and a binary outcome.* Int J Epidemiol, 2014. **43**(3): p. 922-9.

59. Matschinsky, F.M., B. Glaser, and M.A. Magnuson, *Pancreatic beta-cell glucokinase: closing the gap between theoretical concepts and experimental realities.* Diabetes, 1998. **47**(3): p. 307-15.

60. Burdon, K.P., et al., *Association of protein tyrosine phosphatase-N1 polymorphisms with coronary calcified plaque in the Diabetes Heart Study.* Diabetes, 2006. **55**(3): p. 651-8.

61. Gu, P., et al., *Protein tyrosine phosphatase 1B gene polymorphisms and essential hypertension: a case-control study in Chinese population.* J Endocrinol Invest, 2010. **33**(7): p. 483-8.

62. Olivier, M., et al., *Single nucleotide polymorphisms in protein tyrosine phosphatase 1beta (PTPN1) are associated with essential hypertension and obesity.* Hum Mol Genet, 2004. **13**(17): p. 1885-92.

63. Bonetti, S., et al., *Variants of GCKR affect both beta-cell and kidney function in patients with newly diagnosed type 2 diabetes: the Verona newly diagnosed type 2 diabetes study 2.* Diabetes Care, 2011. **34**(5): p. 1205-10.

64. Mohas, M., et al., *GCKR gene functional variants in type 2 diabetes and metabolic syndrome: do the rare variants associate with increased carotid intima-media thickness?* Cardiovasc Diabetol, 2010. **9**: p. 79.

65. Yu, X.H., et al., *ABCG5/ABCG8 in cholesterol excretion and atherosclerosis.* Clin Chim Acta, 2014. **428**: p. 82-8.

66. *Large-scale gene-centric analysis identifies novel variants for coronary*

artery disease. PLoS Genet, 2011. **7**(9): p. e1002260.

67. Wu, G., et al., *ABCG5/8 variants are associated with susceptibility to coronary heart disease.* Mol Med Rep, 2014. **9**(6): p. 2512-20.

68. Dobbelsteyn, C.J., et al., *A comparative evaluation of waist circumference, waist-to-hip ratio and body mass index as indicators of cardiovascular risk factors. The Canadian Heart Health Surveys.* Int J Obes Relat Metab Disord, 2001. **25**(5): p. 652-61.

69. Pouliot, M.C., et al., *Waist circumference and abdominal sagittal diameter: best simple anthropometric indexes of abdominal visceral adipose tissue accumulation and related cardiovascular risk in men and women.* Am J Cardiol, 1994. **73**(7): p. 460-8.

70. Lam, B.C.C., et al., *Comparison of Body Mass Index (BMI), Body Adiposity Index (BAI), Waist Circumference (WC), Waist-To-Hip Ratio (WHR) and Waist-To-Height Ratio (WHtR) as Predictors of Cardiovascular Disease Risk Factors in an Adult Population in Singapore.* PLoS ONE, 2015. **10**(4): p. e0122985.

71. Zohreh K, T.S., Mansour R, *Comparison of obesity indexes BMI, WHR and WC in association with Hypertension: results from a Blood Pressure Status Survey in Iran.* Journal of Cardiovascular Disease Research, 2015. **6**(2): p. 72-77.

72. Meisinger, C., et al., *Body fat distribution and risk of type 2 diabetes in the general population: are there differences between men and women? The*

MONICA/KORA Augsburg cohort study. Am J Clin Nutr, 2006. **84**(3): p. 483-9.

73. Fall, T. and E. Ingelsson, *Genome-wide association studies of obesity and metabolic syndrome.* Mol Cell Endocrinol, 2014. **382**(1): p. 740-57.

74. Molarius, A. and J.C. Seidell, *Selection of anthropometric indicators for classification of abdominal fatness--a critical review.* Int J Obes Relat Metab Disord, 1998. **22**(8): p. 719-27.

75. Bouchard, C., G.A. Bray, and V.S. Hubbard, *Basic and clinical aspects of regional fat distribution.* Am J Clin Nutr, 1990. **52**(5): p. 946-50.

76. van der Kooy, K., et al., *Waist-hip ratio is a poor predictor of changes in visceral fat.* Am J Clin Nutr, 1993. **57**(3): p. 327-33.

77. Bravo, P.E., et al., *Leptin and Hypertension in Obesity.* Vascular Health and Risk Management, 2006. **2**(2): p. 163-169.

78. Fitzgerald, M.L., Z. Mujawar, and N. Tamehiro, *ABC Transporters, Atherosclerosis and Inflammation.* Atherosclerosis, 2010. **211**(2): p. 361-370.

79. Biino, G., et al., *Environmental and genetic contribution to hypertension prevalence: data from an epidemiological survey on Sardinian genetic isolates.* PLoS One, 2013. **8**(3): p. e59612.

80. Manolio, T.A., et al., *Finding the missing heritability of complex diseases.* Nature, 2009. **461**(7265): p. 747-53.

Abstract in Korean (국문 초록)

비만과 고혈압의 인과성 연구: 멘델 무작위 및 유전-환경 상호작용 분석

이미리

의학과 예방의학전공

서울대학교 대학원

연구 배경: 고혈압은 심혈관질환의 위험 요인이며 1990년에서 2010년 사이 질병부담은 점차 증가하는 추세이다. 2014년에 전세계적으로 18세 이상의 22% 의 사람이 고혈압으로 진단되었다. 비만은 고혈압의 중요한 원

인이라는 많은 관찰 연구가 발표되어 있으나, 연구의 특성상 측정되지 않은 혼란 요인이 있을 수 있으며 역 인과관계의 가능성 때문에 인과 관계라고 단정할 수 없다. 또한 무작위대조시험도 짧은 연구 기간과 적은 수의 대상자로 연구를 하기 때문에 한계점이 있다. 따라서 인과성을 입증할 수 있는 멘델리안 무작위 분석법(Mendelian randomization)을 이용할 필요성이 있다.

또한, 이전의 전장유전체 연관성분석에 따르면 일부 유전 변이 형은 고혈압과 관련이 있다고 보고되었지만 고혈압 발병에 대한 유전적 기여는 3 % 미만으로 낮았다. 비만은 고혈압의 중요한 원인이므로 고혈압 발생 위험도가 큰 유전체 감수성 그룹을 판별하는 것은 중요한 일이다. 본 연구의 목표는 첫째, 비만이 고혈압에 미치는 인과 관계를 평가하기 위해 멘델리안

무작위 분석법 (MR)을 사용하였다. 둘째, 우리는 고혈압 발생에 대한 유전자 – 비만 상호 작용을 분석했다.

연구 방법: 첫째, MR 분석은 2001 년부터 2013 년까지 안성 및 안산의 8832 명의 성인 (40-69 세) 에 대한 코호트 연구 에서 수행되었다. 우리는 기초 자료 고혈압과 10년동안 새로 진단 된 고혈압을 사용했다. 체질량 지수에 대한 유전 위험 점수(BMI GRS)를 도구 변수(IV)로 사용하여 비만과 고혈압 간의 인과 관계를 측정하였다. 인과 관계 확률 (OR)의 IV 추정치는 Wald ratio estimator를 사용하여 구한 다음 지수로 표시되어 결과를 OR로 표현한다. 또한 인과적 위험비(HR)의 IV 추정치도 Wald ratio estimator을 사용하였다.

둘째, 상호 작용 연구에서, 우리는 비만 변수로 체질량 지수 (BMI), 허

리 둘레 대 엉덩이 둘레 (WHR) 및 허리 둘레 (WC)을 사용하였다. 또한 기초검사에서 고혈압이 아닌 사람을 대상으로 했다. 우리는 비만과 고혈압 사이의 경로와 관련된 3608 SNP를 선정하고 상호 작용을 위해 1 자유도 (1df) 및 2 자유도 (2df) 테스트를 수행하였다.

결과: 첫째, 연령, 성별, 연구 지역, 교육, 흡연 및 현재의 음주를 보정한 모델을 이용하였고 체질량 지수 (BMI)의 1kg/m^2 증가에 따라 고혈압 교차비(OR)는 1.19, 95 %, 신뢰 구간 (CI) 는 1.17−1.21이었다. 선형 회귀 분석을 통해 유전체 검사를 통해 BMI와 연관된 6 가지 SNP (P 값 <1.0 × 10^{-5})를 선택하고 6 가지 유전적 위험 점수 (GRS)를 만들었다. 우리는 BMI GRS의 표준 편차가 증가 할 때마다 고혈압 위험이 6 ~ 7 % (OR : 1.06 ~ 1.07) 증가하였다. (모든 P 값 <0.05). BMI GRS를 IV로

사용하여, BMI와 고혈압 사이의 인과 관계를 발견했다 (OR : 1.16−1.30, 모든 P 값 <0.05). 민감도 분석에서, 기초 자료 고혈압만을 가지고 한 분석은 인과관계를 보였으나, 10년동안 새로 발생한 고혈압 발생자만을 가지고 한 분석에서는 인과관계를 입증할 수가 없었다.

둘째, 상호 작용 연구에서 우리는 고혈압 발생에 대한 4 가지 유의한 SNPs(WHR과 PTPN1의 rs6020611과 rs754118, GCKR의 WC와 rs3817588, ABCG5의 rs1864815)을 발견했다 (1df P <0.01, 2df P <2 × 10^{-6}). 유의한 SNP 값을 합산하여 유전 위험 점수 (GRS)를 계산했다. 고혈압을 설명하는 BMI, WC 및 WHR의 기여 비율의 증가는 가중된 유전 위험 점수 (WGRS)가 낮은 점수에서 높은 점수로 변할 때 각각 0.90 %, 3.82 % 및 2.65 %로 증가 하였다.

결론 : 멘델리안 무작위 분석법을 사용하여, 비만은 고혈압과 인과 관계가 있음을 발견했다. 이 정보는 비만 감소 프로그램이 고혈압 발병률을 감소시킬 것이라는 증거를 뒷받침하며 중요한 공중 보건 영향을 미칠 것으로 기대가 된다. 그리고 우리는 특정 SNP이 고혈압 발병에서 비만과 유의하게 상호 작용한다는 것을 발견했다. 우리의 연구는 유전적 소인 자체의 기여보다 비만과의 상호 작용에 의한 고혈압 발병에 더 기여한다는 것을 보여주었다.

주요어: 멘델 무작위 분석법, 유전자-환경 상호작용, 비만, 고혈압

학번: 2012-31149