A bidirectional causal association between type 2 diabetes and hypertension based on the life course approach

Jooeun Jeon

The Graduate School

Yonsei University

Department of Public Health

A bidirectional causal association between type 2 diabetes and hypertension based on the life course approach

A Dissertation Submitted to
the Department of Public Health
and the Graduate School of Yonsei University
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy in Public Health

Jooeun Jeon

December 2022

This certifies that the Dissertation of ***Jooeun Jeon*** is approved.

Sun Ha Jee: Thesis Supervisor

Chung Mo Nam: Thesis Committee Member #1

Heejin Kimm: Thesis Committee Member #2

Keum Ji Jung: Thesis Committee Member #3

Ji Young Lee Thesis Committee Member #4

The Graduate School

Yonsei University

December 2022

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

A bidirectional causal association between type 2 diabetes and hypertension based on the life course approach

Jooeun Jeon

Department of Public Health

The Graduate School

Yonsei University

(Supervised by Professor Sun Ha Jee)

Background: The positive relationship between type 2 diabetes (T2D) and hypertension has been proved in a number of large observational studies. These observational studies were limited in confirming causal relationships because of the potential confounding biases and reverse causality. There was only one previous Mendelian randomization (MR) study for the bidirectional causal relationship between T2D and hypertension was conducted, it had ethnical limitations. This study aimed to conduct improved MR study in a Korean population-based longitudinal cohort study and investigate the bidirectional causal relations of fasting blood sugar (FBS) levels with systolic and diastolic blood pressure (BP) using MR analysis, and validate the bidirectional causal association based on life course approach.

Methods: Five MR methods were applied, including the two-stage least squares (2SLS) regression method, inverse-variance weighted (IVW) method, and 2 median-based methods (simple and weighted), MR-Egger was used to assess the bidirectional causal association. The weighted genetic risk score (wGRS) for genetically instrumented FBS and systolic blood pressure (SBP) was constructed using 91 and 68 single nucleotide polymorphisms (SNP) extracted from the GWAS of the large Korean biobank. The p-value cutoff was set at $<1.0\times10^{-8}$ based on multiple linear regression. A trajectory analyses was performed to estimate how

much genetically determined FBS or SBP value estimated from IVs affects future T2D or hypertension incidents. the Cox proportional hazard models were conducted to assess the association analyses between wGRS and future T2D or hypertension in a general healthy population. To evaluate the association analyses between trajectories for genetically determined FBS or SBP value and future T2D or hypertension in a general healthy population, the Cox proportional hazard models were performed.

Results: MR analysis using the two-stage least squares regression method adjusted for age and sex showed that FBS elevation by 10 mg/dL due to our genetic variants was associated with an increased SBP of 1.63 mm/Hg (p=0.005), and genetically determined elevation of SBP by 10 mm/Hg was associated with an FBS increase of 11.39 mg/dL (p<0.0001). Using the MR-Egger method, when the FBS was 1 mg/dL higher genetically, it was associated with a higher SBP of 0.20 mm/Hg (p=0.005, p for intercept=0.823). Meanwhile, an elevated SBP of 1 mm/Hg genetically was associated with an increased FBS of 1.08 mg/dL, and a significant intercept p-value was demonstrated (p<.0001 , p for intercept=0.001). However, after omitting only one outlier (rs671, which has a strong relationship with alcohol drink), the significance for horizontal pleiotropy resolved. A distinct FBS / SBP trajectory (controlled and uncontrolled groups) over time was confirmed using latent group

trajectory analysis after selecting the healthy population at baseline without T2D and hypertension. Subsequently, the incidents of hypertension / T2D were evaluated according to each FBS / SBP trajectory using Cox proportional hazard regression. There was no significant difference between the FBS uncontrolled and controlled groups after adjusting for covariates including antidiabetic medications. Conversely, a significantly higher risk of increased SBP was detected in the uncontrolled group relative to the controlled group (HR=1.27, 95%CI: 1.16-1.38 after adjusting for covariates including antihypertensive medications.

Conclusion: A bidirectional causal association between fasting blood sugar level and systolic blood pressure in the Korean general population was identified based on the life course approach. In the future, elaborative large biobank studies including countless genetic variants and different environmental interactions are needed to validate the bidirectional causal association between FBS and SBP using the life course approach.

Keywords: Genome-wide association study, Single nucleotide polymorphism, Mendelian randomization, Type 2 diabetes, Hypertension

I. INTRODUCTION

Hypertension is reported in more than two-thirds of type 2 diabetes (T2D) patients [1]. Hypertension and T2D are two major components of the global disease burden and often coexist [1]. According to the World Health Organization (WHO), arterial hypertension and T2D are the two most common cardiovascular risk factors in the global population after obesity, and Burden for these two diseases is significant worldwide [2, 3]. In the case of Korea, as of 2020, the prevalence of hypertension was 29% and diabetes was 13.9%, which increased by 5.3% and 3.6%, respectively, over the past 10 years [4].

Many previous prospective studies have linked T2D to an increased risk of hypertension, and evidence of a quantitative relationship between blood pressure (BP) and T2D incidence has been reported [5-7]. Despite each being an independent cardiovascular risk factor, hypertension and T2D often coexist in the same patient. This coexistence doubles the risk of other non-communicable chronic disease i.e.chronic kidney disease in patients [8, 9]. One epidemiological study showed T2D at baseline was a significant predictor of incident hypertension independently of sex, age, body mass index, and familial diabetes mellitus [10].

Although the one-way ssociation between hypertension and T2D has been derived by many researchers through observational studies, but reliable

quantification of the associations between hypertension and the risk of T2D is lacking due to the complex shared mechanisms, multiple environmental factors, the potential bias introduced by unmeasured confounding factors, and potential reverse associations[5-7, 11]. In order to explore not only the one-way association but also the two-way association, a more sophisticated methodology needs to be developed. Research and further understanding of the bidirectional relationship between these two diseases will help prevent future diseases and contribute to treatment, which are the main goals of the global health system.

For this reason, recent attempts have been made to clarify the relationship between hypertension and T2D using MR analysis , and some meaningful results have been obtained [12, 13]. Only one study analyzed the bidirectional causal relationship between hypertension and T2D. This recent bidirectional MR analysis that assessed the causal relationship between hypertension and T2D was performed using hundreds of SNPs from the UK Biobank, which comprised the genotypes of over 300,000 adults. The authors detected causality and significant pleiotropy in the hypertension-T2D relationship [13]. This previous study was limited to the European population. Moreover, it is necessary to further elucidate the complex association including pleiotropy and other behavior covariates, and the causal effect of T2D on the development of hypertension.

Meanwhile, trajectory analysis based on life-course approach has been noticed in epidemiology. It is possible to determine the variation of repeat measured risk factors based on different aspects over time. In addition, beyond the limitations of the study considering only the single point measured exposure, the risk of disease can be grasped in a more diverse way by considering changes of other risk factors likes behavior variables.

Therefore, the main focus of this study is to improve the analysis method for the bidirectional causal relationship between diabetes and hypertension based on previous studies targeting Western population to derive results in Koreans. Methodological improvements include extending the causal relationship to the life-course concept. This present study conducted MR for Koreans on the causal relationship between fasting blood sugar (FBS) and blood pressure (BP) using repeat measured follow-up data. Furthermore, the effects of distinct patterns of genetically predicted FBS and SBP trajectories on the predictive occurrence of T2D or hypertension were visible depending on the trajectory analyses performed to determine the changes in genetically determined FBS and SBP.

II. MATERIALS AND METHODS

1. The study population and ascertainment

1-1. Participants in the mendelian randomization analysis

The present study population belongs to the Korean genome and epidemiology study (KoGES), Ansung–Ansan cohort, which included a total of 10,030 consented participants, aged 40 to 69 years, recruited from two communities (Ansung, n=5,018 and Ansan, n=5,012) in the Gyeonggi-do province, South Korea, between 2001 and 2016 [14]. Survey data have been following up every 2 years to collect questionnaires, anthropometric / biomarker measurements, blood sampling, and urine tests (at the 8th visit, the follow-up rate=61.4%). DNA Genotype data were linked to phenotype data from KoGES cohort provided by the Center for Genome Science, Korea National Institute of Health. DNA samples were separated and extracted from each peripheral blood of the participants [14]. Out of baseline participants, 8,836 people who have available Genome-wide single nucleotide polymorphism (SNP) data. Among them, 326 people were omitted due to having outliers or missing information of phenotype data. Finally, 8,510 participants were selected for MR analysis in this present study (**Figure 1**).

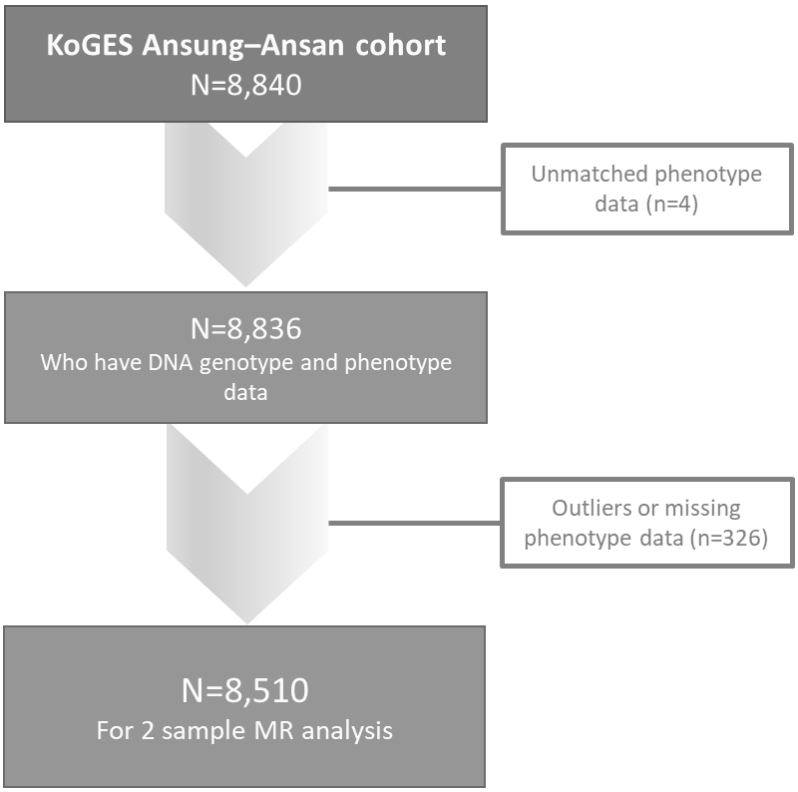


Figure 1. The study population for mendelian randomization analysis

1-2. Participants in the association analysis

The data for association analysis were based on the life course approach reconstructed according to the research objective. And also, the association analysis was divided to two types according to the research objective. First association analysis was conducted to identified direct association between genetic instrument variants for FBS or SBP and incidences of T2D or hypertension. Second association analysis was performed to extend and replicate based on the life course approach the causal association confirmed MR analysis above.

Of the 8,510 participants included in the MR analysis, only newly diagnosed patients with T2D and hypertension were included during the second to eighth visits. Moreover, patients who reported a history of T2D (n=892) or hypertension (n=1,293) or stroke (n=34) in the baseline survey were excluded. Consequently, 6,278 participants were included to the Cox proportional hazard regression model to assess the association with subsequent T2D or hypertension. T2D was defined as serum fasting blood sugar levels above 126 mg/dL, hemoglobin A1c above 6.5%, a history of T2D diagnosis, taking insulin drugs, or taking antidiabetic medications during the first to eighth visits. Hypertension was defined as a systolic blood pressure of ≥140 (mm/Hg), or a diastolic blood pressure of ≥90 (mm/Hg), or the use of blood pressure-lowering medication during follow-ups (**Figure 1**).

Follow-ups for T2D or hypertension cases were conducted from the 2nd visit to the onset of T2D or hypertension. Follow-ups for other participants were conducted from 1st visit to December 31, 2017. For patients with T2D or hypertension cases whose dates of T2D or hypertension diagnosis (year, month, and day) could not be ascertained, T2D or hypertension was defined autonomously because of data attribute. Among 6,278 participants, the number of subsequent T2D was 3,220 people (51.29%) and subsequent hypertension was 4,323 people (68.86%). And also, mean of follow-up time for T2D or hypertension were 10.59 years (66493.62 person-years) for T2D and 8.70 years (54633.00 person-years) for hypertension. During the follow-up period, 2933 people were co-exist cases with both hypertension and T2D, accounting for 67.8% of 4,323 hypertensive patients and 91.1% of 3,220 diabetic patients.

The parent study of this present study was approved by the Ethics Committee of the Korean Center for Disease Control and the Institutional Review Boards of the Korea University Ansan Hospital and the Ajou University School of Medicine. The Institutional Review Board (IRB) of Human Research of Yonsei University in this study approved the research (IRB number 4-2022-1371). Review board requirement for written informed consent was waived because this study used an anonymous dataset.

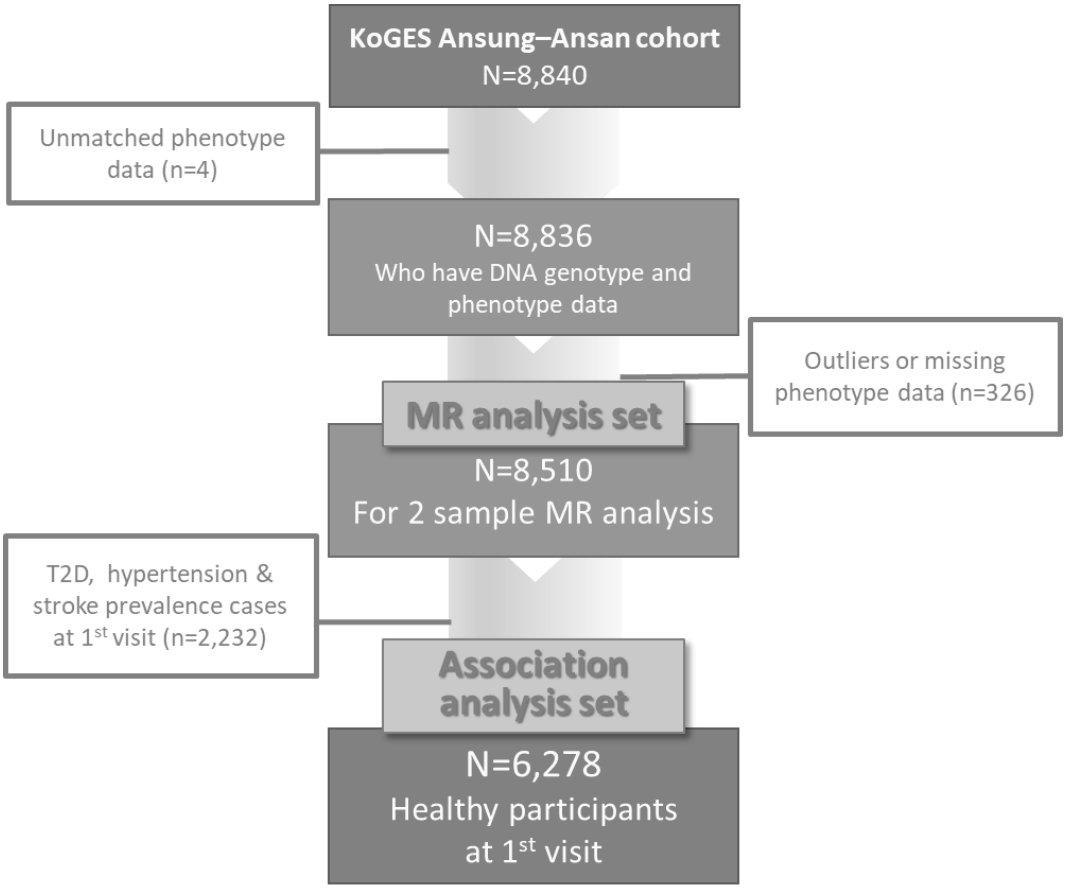


Figure 2. Flow chart for study population

2. Genotyping and genetic instrument variants selection

A genome-wide association study (GWAS) was performed using by the Korean CHIP genetic data (from Korean cancer prevent study-II biobank, KCPS-II biobank) to identify SNPs associated with FBS levels or BP (SBP), adjusting for age and sex [15]. All variants applied to the following exclusion criteria for quality control process [16, 17]: (i) subjects with <5% missing genotype were included in the analysis, (ii) markers showing significant deviations from the Hardy-Weinberg equilibrium ($P<1.0\times10^{-4}$), (iii) genotyping accuracy less than 96–99%, and (iv) minor allele frequency <0.01. After the quality control (QC) evaluations, the remaining 317,290 variants were subjected to further analyses. Based on previous GWAS analysis on FBS and SBP, 5,708 variants for FBS levels and 5,089 variants for SBP with a statistical cut-off p-value ($<1.0\times10^{-8}$) were remained (**Figure 3**). Subsequently, the linkage disequilibrium (LD) clumping algorithm was used to identify the independent SNPs for use as instrumental variables with an R2 threshold below 0.03 and a p-value of $<5.0e^{-8}$. After excluding the correlated SNPs using the clumping algorithm, 154 variants for FBS levels and 119 for SBP levels were initially determined by GWAS using data from the Korean CHIP genetic data.

Moreover, genotyping for the dataset with 8,840 KoGES Ansung–Ansan cohort subjects was performed using the Affymetrix Genome-Wide Human SNP

Array 5.0 and Affymetrix Axiom KORV1.1-96 Array (Affymetrix, Santa Clara, CA, USA), respectively, which was performed by DNAlink Inc. (Seoul, Korea) as previously described [14]. For GWAS using by KoGES Ansung–Ansan cohort, Imputed SNPs with a high genotype information content (info >0.5) based on The HapMap phased genotype information of Japanese individuals from Tokyo, Japan (JPT) and unrelated Han Chinese individuals from Beijing, China (CHB) (build 36 release 22) were used in this present study [18]. The same exclusion criteria as those mentioned earlier were applied to the imputed SNPs and genotyped SNPs. In total, 154 variants for FBS levels and 119 variants for SBP measurements from the GWAS of the KCPS-II were applied to KoGES as imputed and genotyped data. Consequently, 91 variants for FBS levels and 68 variants for SBP measurements remained after matching the KoGES genotyping dataset and selecting the genetic instrument variants for the mendelian randomization analysis (**Figure 4**).

A weighted genetic risk score (wGRS) for each phenotype (FBS and SBP) was constructed using the following formula: $wGRS = \beta1 \times SNP1 + \beta2 \times SNP2 + \ldots + \beta n \times SNPn$. In this formula, SNPn is the number of risk alleles and βn is the corresponding effect size associated with the SNPn obtained from previous GWAS analyses of the KCPS-II biobank [19]. To achieve normal distribution for wGRS, the data was conducted reverse beta correction (**Figure 5**).

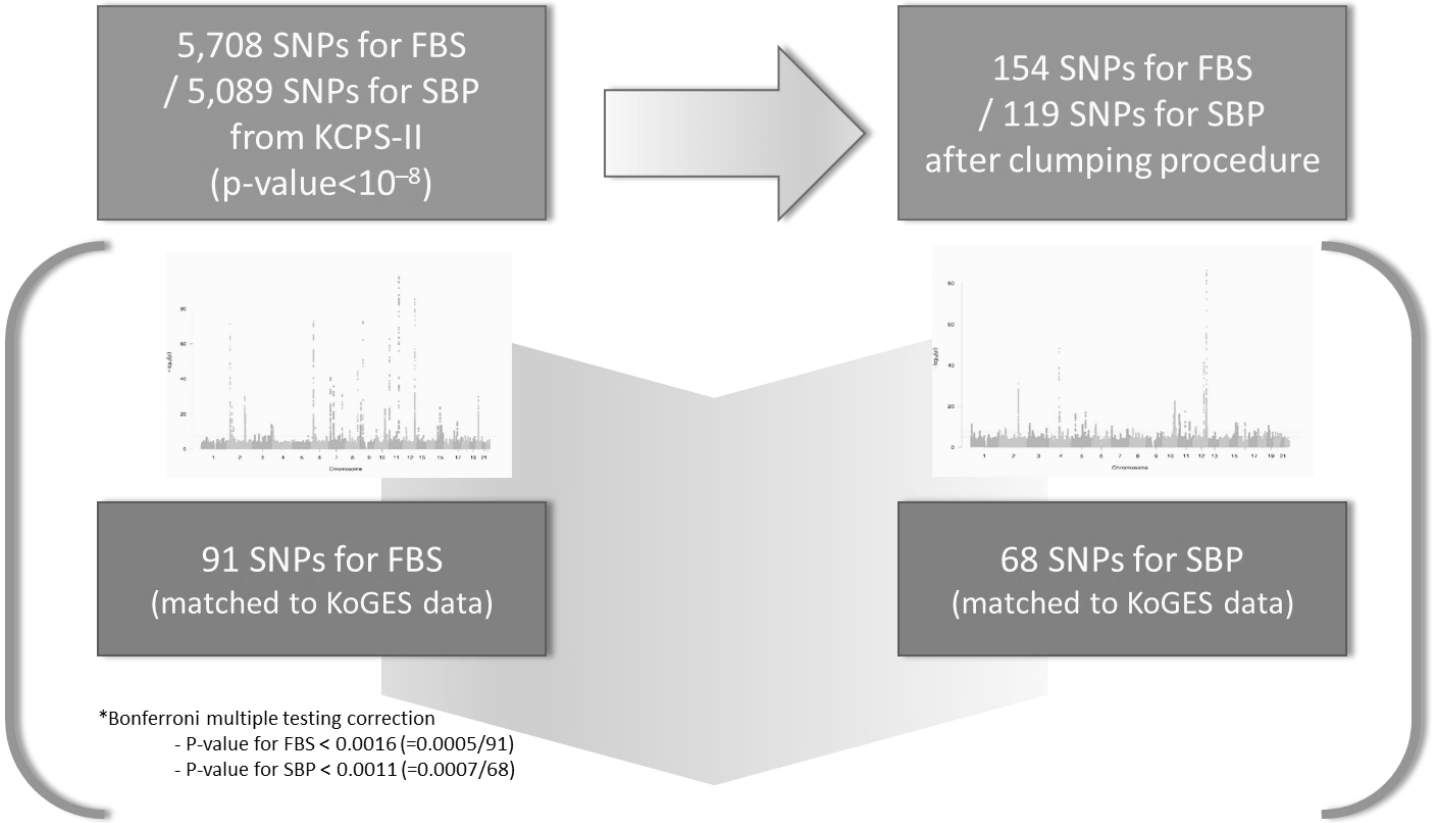


Figure 3. Genetic instrument variants selection procedures

A. fasting glucose levels **B. systolic blood pressure**

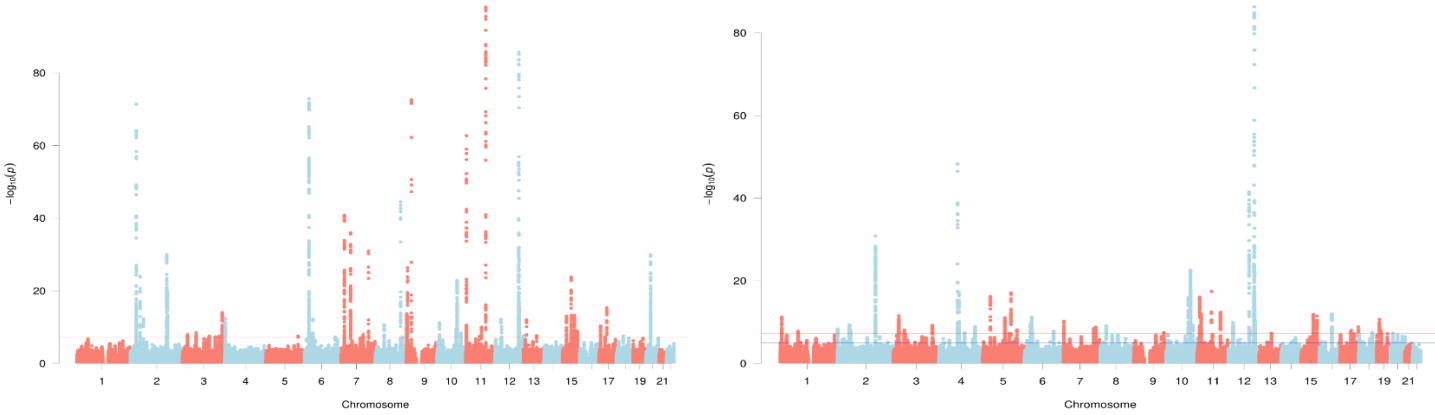


Figure 4. Manhattan plots for fasting glucose levels and systolic blood pressure from KCPS-II

Plot for fasting glucose levels and systolic blood pressure showing the –log10 transformed p-value of SNPs.

P values of the y-axis were adjusted for age and sex

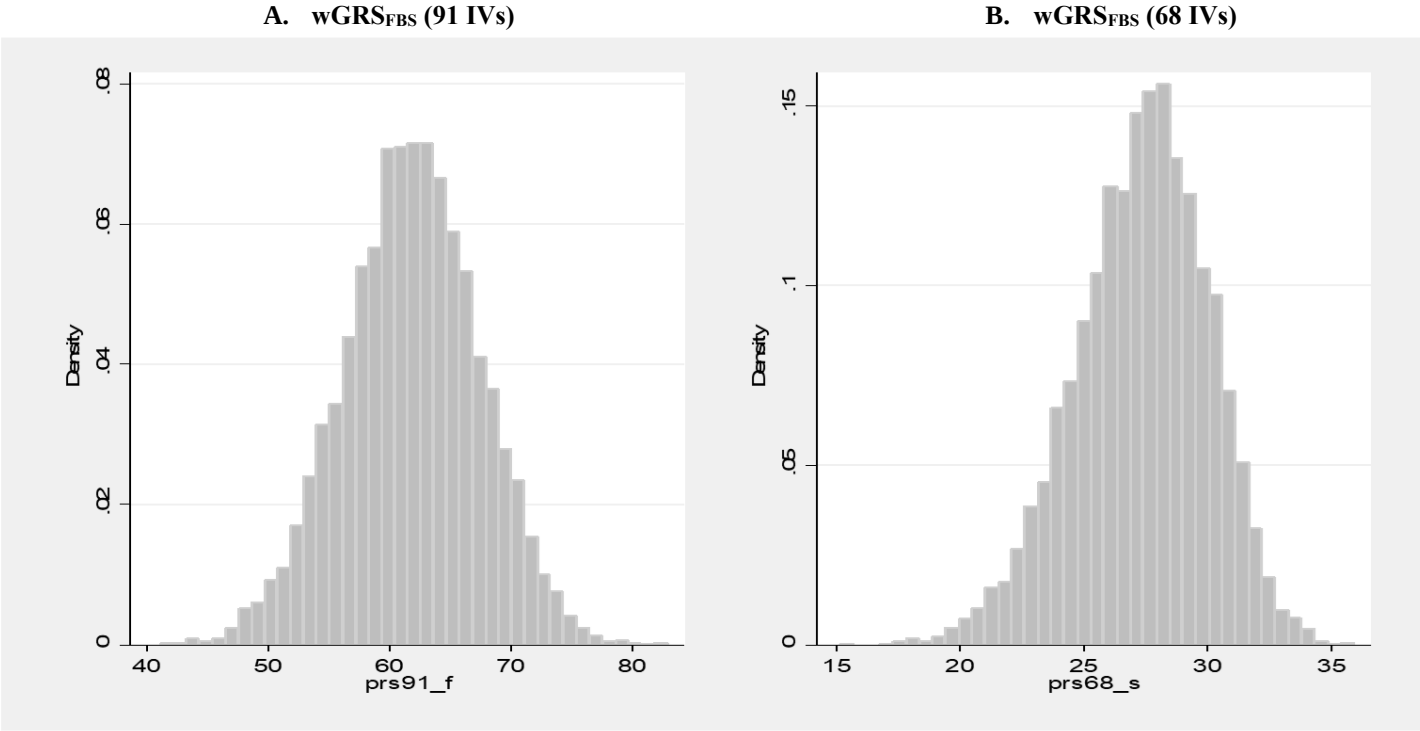


Figure 5. Histograms of weighted genetic risk score for fasting blood sugar or systolic blood pressure levels

3. Statistical methods

3-1. Bidirectional mendelian randomization analysis

Recently, many researchers have shown interest in causal inference, and many methodologies related to this have emerged. The inference of causality from observational evidence may be problematic, as observational studies frequently include confounding factors or reverse causation for the identification of associations between exposure and outcome. To overcome the limitations of randomized controlled trials (RCTs), Mendelian randomization (MR) analysis has been utilized as an alternative [20-22]. MR based on the assumption that genetic variants assigned prior to conception are randomly allocated according to Mendel's second law [23, 24]. MR is a method of using measured variation in genes of known function to examine the causal effect of a modifiable exposure on disease in observational studies. MR can provide more credible estimates of the causal effect of a risk factor on an outcome than those obtained in observational studies [22].

MR uses a genetic variant as a proxy for a risk factor. Therefore, a successful MR study is dependent on the appropriate selection of a genetic instrument variable (IV). [22]. MR can estimate causal effects where exposure and outcome data from different samples exist. IVs are typically identified using GWAS. To validate a genetic variant as a valid IV for causal inference in an MR analysis, three central

IV assumptions must be satisfied [20-24] (Figure 2): (1) The genetic variant must be directly associated with the exposure. (2) The genetic variant must be independent of unmeasured confounders known to obscure the connection between the exposure and the effect. The genetic variant must show no effect , other than through the exposure. [22]. The horizontal pleiotropic pathways induce bias in the direction of the pleiotropic association and exclude the associations that violate the IV assumptions, especially assumption 3. Current MR methodologies that consider pleiotropic pathways have been established by the emergence of large-scale biobank projects. When several genetic variants are correlated with a specific exposure, summary level MR methods using a summarized data can typically evaluate the set of Wald ratios within a meta-analytic framework using various methods including the inverse-variance weighted (IVW) method, MR-Egger regression, and weighted medians [25-31].

1) A single genetic instrument - wald ratio estimator

The Wall ratio is a causal estimate for a single-genetic variant, which is calculated by dividing the genetic variant-outcome association by the genetic variant exposure association when a single genetic variant is available. This can be easily seen as the change in the outcome of a unit change in exposure [29-32].

2) Summary level MR method

(1) Inverse-variance weighting (IVW) method

The IVW test, is a weighted average of the causal effects of genetic variants. When pleiotropic pathways exist on several genetic variants correlating with a specific exposure, the Wald ratio approach can be generalized through a meta-analysis process. In particular, the causal effect estimates of each genetic variant are combined in an IVW meta-analysis framework [22, 27, 32].

(2) The MR-Egger test

The MR-Egger method is one of many methods have been devised for defining and correcting breaches of assumptions, when some of genetic variants selected are invalid instruments. When the size of such pleiotropic effects is independent of the size of the effect of the genetic variant on the risk factor, the MR-Egger method permits a pleiotropic effect of one or more genetic variants. In the regression of MR-Egger method, the intercept can be viewed as an estimate of the (horizontal) average pleiotropic effect of genetic variants and the slope of the regression as the corrected causal effect. Generally, the MR-Egger method is statistically less powerful than the IVW method. The MR-Egger regression replaces the second and

third IV assumptions with the "instrument strength independent on direct effect (InSIDE)" assumption [25, 28].

(3) Simple median estimator

The IVW estimate is an efficient analysis method when all genetic variants are valid IVs. Unfortunately, it will be biased even if only one genetic variant is invalid. If up to (but not including) 50% of the genetic variants are invalid, the median ratio estimate may be used as a simple estimator [21, 26, 32].

(4) Weighted median estimator

If up to 50% of the genetic variants are invalid, then a causal effect may be estimated as the median of the weighted ratio estimates using the reciprocal of the variance of the ratio estimate as weights. The InSIDE assumption is not needed and violations of the second and third IV assumptions are permitted. In contrast to the MR-Egger method and the simple median estimator, the weighted median estimator has the benefit of preserving greater precision in the estimates [21, 26, 32].

3-2. Latent class trajectory analysis

Trajectory analysis that utilizes a multinomial mixture modeling strategy is useful for identifying relatively homogeneous clusters of change trajectories over time in repeated observations of analytic subjects [33-35]. In trajectory analyses, the longitudinal follow-up data are modeled by having the parameter depending on time. The basic assumption is that time-dependent covariates can also directly affect the observed behavior and all individuals in the study sample come from a single population Therefore, one (average) trajectory should adequately describe the developmental pattern of the sample [36].

Trajectory models were estimated with 2-5 trajectories by assuming linear, quadratic, and cubic patterns of change in FBS or SBP over time using appropriate statistical tool. In this trajectory analysis, each participant was assigned to the class for which his/her posterior probability was the highest under specific conditions. To ensure that all obtained classes were of clinically and statistically meaningful size, the conditions that each class should include at least 5% of participants and the mean posterior probability of each class should be higher than 75% were imposed. As recommended, the best-fitting model was selected by comparing the BIC indices associated with automatically calculated and averaged posterior probabilities of the group membership in each latent class for each participant [33-36].

Of the 8,510 participants included in the initial GWAS and Mendelian randomization analysis, 6,278 participants without past disease history of T2D (n=892) or hypertension (n=1,293) or stroke (n=34) in the baseline survey were included to cox proportional hazard regression model to assess association with subsequent T2D or hypertension. These all had wGRS made by FBS levels or SBP levels relating genetic variants.

3-3. Statistical Analysis

GWAS were performed using PLINK, version 1.9 (http://pngu.mgh.harvard.edu/purcell/ plink/) to identify SNPs associated with FBS levels or SBP measurements via linear regression analyses with an additive model. Age and sex were fitted as fixed covariates, and the cutoff p-value of $<5\times10^{-8}$ was used to indicate genome-wide significance. To assess bidirectional causalities of variants with FBS levels and SBP, a 2-stage least squares (2SLS) regression model was performed using by STATA/IC 13.1 (Stata Corp LP, College Station, TX, USA) with wGRS including 91 variants for FBS levels and 68 variants for SBP. Additionally, not only bidirectional pathway between FBS and SBP, also the other causal relationship to other metabolic components including hemoglobin A1c (HbA1c), total cholesterol levels and triglyceride (TG) levels were evaluated. To test for evidence of pleiotropy, the sensitivity of the mendelian randomization analysis was conducted using R version 4.1.2 software (http://www.r-project.org/) with beta coefficients (the estimates resulting from a regression analysis in GWAS), standard error (SE), or SE of 91 variants for FBS and 68 variants for SBP as instrument variables.

In this study, the general characteristics were expressed as means ± standard deviation (SD) or frequency (percentage). T-test and chi-square test were performed to assess group differences (by gender or trajectories). To evaluate the association analyses between the weighted genetic risk score (wGRS) based on genetic instrument variants passed MR analysis and future T2D (i.e., diagnosed by a physician or treated using anti-diabetic drugs) or hypertension (i.e., diagnosed by a physician or treated with anti-hypertensive drugs) in a healthy general population, the Cox proportional hazard model, adjusted for age, sex, body mass index (BMI), smoking behavior (non-smoker, ex-smoker, and current-smoker), alcohol drinking (non-drinker or ever-drinker) and regular exercise (yes or no) and antidiabetic (for hypertension)/antihypertensive medication (for T2D) was applied.

To further verify whether the strong results in MR analysis are also shown in life-course approach-based association analysis, a trajectory analyses was performed to estimate how much genetically determined FBS or SBP value estimated from IVs affects future T2D or hypertension incidents, as in the 2SLS regression model. The latent class trajectory analyses were conducted to evaluate the pattern of changes in genetically determined FBS levels or SBP measurements over time in the healthy population (n=6,278) who inherited major variants including wGRS that passed the MR analysis.

Predicted FBS or SBP levels were obtained using by linear regression model between wGRS and FBS or SBP measurements. In the trajectory analysis process, quadratic or cubic patterns of change in predicted FBS or SBP over time were evaluated using the SAS PROC TRAJ package (SAS Institute, Inc., Cary, NC, USA). Additionally, to examining the associations of subsequent T2D (i.e., diagnosed by a physician/treated with anti-diabetic drugs) or subsequent hypertension (i.e., diagnosed by a physician/treated with anti-hypertensive drugs) in each trajectory pattern, the Cox proportional hazard model, adjusted for age, sex, BMI, smoking behavior, alcohol drinking, regular exercise and wGRS (including 91 variants for FBS levels or 68 variants for SBP) were conducted using SAS (version 9.4; SAS Institute, Cary, NC, USA) Quantitative interactions by sex were assessed on additive and multiplicative scales. Additive scales were assessed using the relative excess risk due to interaction (RERI) [37, 38]. All statistical tests were two-sided, and the statistical significance was determined as $p < 0.05$.

III. RESULTS

PART I. A bidirectional causal association between FBS (T2D) and SBP (hypertension)

1. Genetic instrument variants

Among 154 variants for FBS levels and 119 variants for SBP measurements from the GWAS of the KCPS-II, 91 variants for FBS and 68 variants for SBP were selected as genetic instrument variants after matching KoGES imputed genotyping dataset and determining a statistical cut-off p-value at $<1.0\times10\text{-}8$ based on multiple linear regression models, adjusted for age and sex.

Table 1 displays the list of 91 genetic variants for FBS. Diverse genetic susceptibility variants relating to 91 genetic instrumented variants for FBS included the well-known rs10440833 related to T2D (located at the CDKAL1 gene). **Table 2** displays the list of 68 genetic variants for SBP. Various chromosomes were included in this list for SBP. Specifically, aldehyde dehydrogenase type 2 (ALDH2) rs671 polymorphism was included in both FBS or SBP.

Table 1. Selected genetic variants for fasting glucose levels (N=91)

No	SNP	CHR	BP	Reference allele	Alternative allele	GWAS					
						KCPS-II			KoGES		
						beta	SE	P	beta	SE	P
1	rs10124848	9	623485	T	A	0.67489	0.08819	1.97625E-14	-0.05505	0.10399	0.596503
2	rs10259649	7	44219705	T	C	0.92814	0.10501	9.79149E-19	0.19952	0.12280	0.104204
3	**rs10440833**	**6**	**20688121**	**T**	**A**	**1.21820**	**0.06709**	**1.35108E-73**	**0.02687**	**0.07844**	**0.7319**
4	rs10814921	9	4307572	T	C	-0.44064	0.06727	5.77025E-11	0.09565	0.07876	0.22458
5	rs10830964	11	92719681	C	T	-0.80733	0.07710	1.19966E-25	-0.05723	0.09159	0.532086
6	rs10849920	12	1.11E+08	C	T	0.47766	0.06780	1.85881E-12	0.32684	0.07915	3.64E-05
7	rs10955807	8	1.18E+08	A	G	0.57419	0.06707	1.12299E-17	0.03293	0.07838	0.674445
8	rs10965241	9	22129594	G	C	0.78648	0.11748	2.17358E-11	-0.17465	0.13778	0.204951
9	rs10965250	9	22133284	G	A	-1.22340	0.06751	2.58177E-73	-0.10269	0.07915	0.194516
10	rs10965251	9	22134029	G	A	-0.93675	0.12931	4.36984E-13	0.08368	0.14656	0.568021
11	rs11020106	11	92667147	T	A	-0.68198	0.06700	2.51849E-24	-0.08943	0.07879	0.256335
12	rs11065836	12	1.12E+08	G	A	-0.53834	0.06765	1.75874E-15	-0.29544	0.07941	0.000199
13	rs11071655	15	62427973	T	C	-0.43768	0.06741	8.43155E-11	-0.00070	0.07864	0.992883
14	rs11187078	10	94340705	G	C	0.69814	0.06973	1.38272E-23	-0.09938	0.08162	0.223363
15	rs11187146	10	94478355	C	G	0.52254	0.07136	2.4467E-13	-0.09138	0.08299	0.270827
16	rs11187165	10	94515985	T	C	0.81087	0.12577	1.14262E-10	-0.05755	0.14531	0.692048
17	rs113748381	17	6953155	G	A	0.74878	0.11388	4.8792E-11	0.22172	0.12977	0.087529
18	rs113767488	7	44214513	T	C	-0.73508	0.07909	1.5046E-20	-0.09204	0.09327	0.323783
19	rs11753021	6	20735394	C	T	-0.58638	0.07455	3.7078E-15	-0.14640	0.08745	0.094125
20	rs12053049	2	1.7E+08	T	C	0.80614	0.07003	1.19171E-30	0.18681	0.08215	0.022965

21	rs12219514	10	94466439	G	A	0.80591	0.09456	1.56658E-17	-0.11745	0.11049	0.287769
22	rs12297293	12	1.13E+08	G	C	-0.55517	0.06886	7.53735E-16	-0.35273	0.08064	1.22E-05
23	rs12472643	2	27739306	C	T	0.73858	0.09960	1.22013E-13	-0.13979	0.11713	0.232713
24	rs1260326	2	27730940	T	C	1.20910	0.06730	4.19189E-72	-0.28932	0.07911	0.000255
25	rs12712928	2	45192080	G	C	0.70970	0.06915	1.05668E-24	-0.01336	0.08089	0.868771
26	rs13266634	8	1.18E+08	C	T	-0.96601	0.06845	3.34705E-45	0.01364	0.08025	0.865025
27	rs13383793	2	45176962	T	C	0.38747	0.07073	4.30447E-08	0.14163	0.08244	0.0858
28	rs1376556	2	1.74E+08	C	G	-0.57386	0.09096	2.81997E-10	-0.07401	0.10490	0.480461
29	rs1377186	18	31523975	T	C	-0.37394	0.06728	2.73599E-08	0.03872	0.07941	0.625802
30	rs142190217	12	1.1E+08	G	A	-0.84611	0.11942	1.39268E-12	-0.39647	0.14927	0.007906
31	rs144934275	5	1.51E+08	G	A	0.73739	0.13288	2.87235E-08	-0.04671	0.15549	0.763865
32	rs1574285	9	4283137	T	G	0.73026	0.06777	4.60297E-27	0.04842	0.07920	0.540947
33	rs1680054	4	1221136	C	T	-0.47746	0.07563	2.73829E-10	-0.02055	0.08904	0.817467
34	rs16940688	12	1.1E+08	G	A	-1.00660	0.12097	8.79808E-17	-0.75716	0.14995	4.44E-07
35	rs17168486	7	14898282	C	T	0.70878	0.06794	1.80603E-25	0.06318	0.07947	0.426624
36	rs1881395	2	27838549	G	A	0.77687	0.07117	9.92294E-28	-0.23136	0.08296	0.00529
37	rs2043880	15	90432526	A	G	0.52258	0.08582	1.13787E-09	0.08127	0.09974	0.415163
38	rs2072134	12	1.13E+08	G	A	-1.32246	0.10521	3.22962E-36	-0.85419	0.12471	7.48E-12
39	rs2072137	12	1.13E+08	T	C	-0.47856	0.06750	1.35008E-12	-0.19040	0.07919	0.016201
40	rs2106464	11	2639233	C	T	-0.66177	0.11227	3.76553E-09	0.05497	0.12414	0.657924
41	rs217554	7	14905933	G	A	-0.54403	0.07461	3.07942E-13	-0.07709	0.08842	0.383273
42	rs2237897	11	2858546	C	T	-1.15337	0.06857	1.98518E-63	-0.06849	0.08079	0.396547
43	rs2239614	7	44143124	C	T	-0.52649	0.06704	4.0886E-15	-0.24179	0.07845	0.002056
44	rs2290203	15	91512067	A	G	-0.40620	0.06681	1.20766E-09	0.14444	0.07887	0.067069
45	rs231361	11	2691500	A	G	-0.56513	0.08479	2.65764E-11	0.01305	0.09898	0.895126

46	rs243018	2	60586707	G	C	-0.50934	0.07056	5.27013E-13	0.03891	0.08276	0.638272
47	rs2466294	8	1.18E+08	G	C	0.62125	0.08181	3.13013E-14	0.13676	0.09704	0.158742
48	rs2497309	10	94483976	T	C	1.13163	0.12271	2.94766E-20	0.04221	0.14320	0.76818
49	rs2815650	10	12558035	G	A	-0.36767	0.06700	4.08243E-08	0.06559	0.07847	0.403188
50	rs2971670	7	44226101	C	T	1.04096	0.08629	1.70136E-33	-0.03708	0.10074	0.712812
51	rs2971672	7	44205906	A	C	0.63990	0.06706	1.41718E-21	0.16303	0.07820	0.0371
52	rs3852527	11	2826603	G	A	0.39989	0.06831	4.82007E-09	0.00574	0.08051	0.943186
53	rs3937435	12	1.13E+08	A	G	-0.61507	0.07230	1.80569E-17	-0.31793	0.08489	0.00018
54	rs4331050	11	92696014	G	T	1.42506	0.06749	7.8039E-99	0.11341	0.07897	0.150982
55	rs4340647	3	23471072	T	G	-0.43069	0.07535	1.0936E-08	0.25290	0.08787	0.004001
56	rs4712530	6	20713914	T	C	0.83610	0.11789	1.32557E-12	-0.05123	0.12950	0.692404
57	rs4731419	7	1.28E+08	T	C	0.46196	0.08310	2.71926E-08	-0.10102	0.09783	0.301824
58	rs4775468	15	62401926	C	T	-0.52517	0.08545	7.95788E-10	-0.07782	0.09984	0.435755
59	rs4886511	15	77448838	T	C	0.51180	0.06799	5.19174E-14	-0.07842	0.07947	0.323761
60	rs4923864	15	40634717	A	G	0.53566	0.07189	9.27205E-14	0.02793	0.08419	0.740043
61	rs55716278	10	94198194	A	G	1.10136	0.14738	7.88357E-14	-0.01286	0.17740	0.942213
62	rs57195659	12	27964928	G	A	-0.50827	0.07084	7.26281E-13	0.01613	0.08286	0.84565
63	rs6048249	20	22660111	C	G	-0.74271	0.10745	4.78514E-12	-0.17270	0.12875	0.179831
64	rs6456354	6	20519390	A	G	-0.57752	0.08995	1.35986E-10	0.12232	0.10554	0.246489
65	rs671	12	1.12E+08	G	A	-1.79427	0.09101	2.05772E-86	-1.17308	0.10835	2.71E-27
66	rs67320261	6	20609451	C	T	-0.71876	0.06860	1.12381E-25	-0.00045	0.07990	0.995547
67	rs6741646	2	27348198	C	T	-0.42715	0.06969	8.85377E-10	0.27789	0.08154	0.000655
68	rs7090695	10	1.13E+08	C	G	-0.39417	0.06706	4.15616E-09	0.01887	0.07926	0.811781
69	rs7161785	15	62395224	G	C	-0.68603	0.06707	1.50798E-24	-0.06254	0.07842	0.42517
70	rs72657615	6	20671084	A	C	-0.73974	0.10431	1.3329E-12	0.17230	0.11905	0.147822

71	rs72832313	6	20741680	T	C	0.63761	0.06865	1.60181E-20	0.12050	0.08051	0.134463
72	rs73016223	3	1.53E+08	T	C	0.40494	0.07419	4.81713E-08	0.12533	0.08693	0.149405
73	rs7314904	12	1.12E+08	G	A	0.56435	0.10098	2.29335E-08	0.22241	0.11813	0.059734
74	rs73199895	12	1.12E+08	G	A	0.39807	0.06967	1.10653E-08	0.15965	0.08237	0.052585
75	rs742761	6	39046655	C	T	-0.58649	0.08160	6.63422E-13	-0.15958	0.09638	0.097793
76	rs74770198	15	62431368	C	G	-0.67734	0.12233	3.07954E-08	-0.08788	0.14250	0.537419
77	rs75628519	12	1.1E+08	A	G	-0.69036	0.12569	3.96708E-08	-0.32905	0.14620	0.024405
78	rs7656416	4	1254535	C	T	-0.51959	0.07122	2.97775E-13	0.03080	0.08390	0.713575
79	rs76924981	6	20572355	G	C	0.93094	0.13913	2.21859E-11	0.01756	0.16397	0.914732
80	rs77466626	11	61631690	C	T	-0.37336	0.06797	3.96266E-08	-0.12348	0.08039	0.12453
81	rs77853892	2	28067559	C	A	-0.59294	0.10249	7.25035E-09	-0.04430	0.11855	0.708635
82	rs7875253	9	4285707	A	C	-0.41135	0.07300	1.7564E-08	0.13505	0.08523	0.113093
83	rs7997912	13	33562505	T	C	0.64650	0.09055	9.40243E-13	0.00079	0.10679	0.994068
84	rs836598	2	1.74E+08	T	C	-0.57690	0.08187	1.83782E-12	-0.11695	0.09504	0.218499
85	rs912175	9	712137	G	C	0.52043	0.09376	2.84627E-08	-0.03806	0.10917	0.727399
86	rs926091	10	89721412	C	T	0.44178	0.07067	4.08702E-10	0.02735	0.08245	0.740067
87	rs932443	6	39042334	T	C	0.40267	0.06851	4.16383E-09	-0.01752	0.08035	0.827394
88	rs9358341	6	20525488	C	A	-0.56315	0.06697	4.19016E-17	0.03718	0.07867	0.636525
89	rs9465844	6	20630472	A	G	-0.62125	0.07115	2.52967E-18	-0.00915	0.08406	0.913344
90	rs9788635	15	62133674	C	T	-0.55222	0.07689	6.89629E-13	-0.08712	0.09035	0.334933
91	rs9842724	3	63804761	C	T	-0.40990	0.06944	3.58346E-09	-0.05187	0.08113	0.522548

Abbreviations: SNP, single nucleotide polymorphisms; CHR, chromosome; GWAS, genome-wide association study; KCPS-II, Korean cancer prevention study-II; KoGES, korean genome and epidemiology study; SE, standard error

Table 2. Selected genetic variants for Systolic blood pressure (N=68)

No	SNP	CHR	BP	Reference allele	Alternative allele	GWAS KCPS2			KoGES		
						beta	SE	P	beta	SE	P
1	rs10190857	2	50678471	G	A	0.301385	0.048327	4.49E-10	-0.09686	0.10431	0.353125
2	rs10434005	4	1.11E+08	G	A	0.264832	0.048189	3.9E-08	0.093422	0.104033	0.369186
3	rs10774611	12	1.11E+08	A	G	0.340194	0.052252	7.5E-11	0.244519	0.112698	0.030035
4	rs10947434	6	33691501	T	G	0.325512	0.058321	2.39E-08	0.123521	0.125655	0.325603
5	rs1106393	10	1.05E+08	C	A	-0.40395	0.051683	5.49E-15	-0.23285	0.111821	0.037317
6	rs11066344	12	1.13E+08	T	A	0.340699	0.05675	1.93E-09	0.497995	0.123145	5.26E-05
7	rs11066453	12	1.13E+08	A	G	-0.96852	0.073024	4E-40	-1.0977	0.159051	5.19E-12
8	rs11072506	15	75052994	G	A	0.278528	0.048947	1.27E-08	0.068959	0.105943	0.515106
9	rs115379475	6	32200681	G	A	-0.40152	0.070775	1.4E-08	-0.37114	0.152851	0.015181
10	rs11870849	17	78411073	C	T	0.430145	0.071051	1.42E-09	0.214328	0.152655	0.160323
11	rs12066994	1	77930043	C	T	-0.274	0.048406	1.51E-08	0.059813	0.104864	0.568417
12	rs12537566	7	1.31E+08	G	C	0.27579	0.048705	1.49E-08	-0.00876	0.10578	0.934011
13	rs12571461	10	95974495	G	A	0.321614	0.05464	3.96E-09	-0.10485	0.117237	0.371122
14	rs12579052	12	90132147	G	A	-0.42854	0.060348	1.24E-12	-0.08796	0.13125	0.502762
15	rs12656497	5	32831939	C	T	-0.41211	0.04931	6.46E-17	-0.04342	0.107017	0.684964
16	rs13139571	4	1.57E+08	C	A	-0.35092	0.057842	1.31E-09	0.238633	0.125437	0.057122
17	rs139037971	19	11518552	G	A	0.407336	0.060801	2.1E-11	0.014802	0.12963	0.909089
18	rs139141104	6	30989021	A	G	0.552844	0.091042	1.26E-09	0.195878	0.195884	0.317327
19	rs1408820	10	96013824	C	T	-0.44323	0.053516	1.22E-16	0.046317	0.115357	0.688048
20	rs141965732	12	1.11E+08	C	T	-0.68197	0.083773	3.96E-16	-1.45706	0.189952	1.73E-14
21	rs144253733	3	1.69E+08	G	A	0.485431	0.078417	6.02E-10	-0.54567	0.169129	0.001255
22	rs1635133	12	1.13E+08	T	C	0.326805	0.048369	1.42E-11	0.239404	0.104556	0.02204
23	rs16998073	4	81184341	A	T	0.747457	0.050772	5.06E-49	0.029437	0.109757	0.788543

24	rs17011002	4	86731385	C	G	0.51326	0.059634	7.59E-18	0.26304	0.12835	0.040427
25	rs17011215	4	86856588	A	G	0.31568	0.05409	5.35E-09	0.128739	0.117181	0.27193
26	rs1750480	10	1.05E+08	T	G	0.377998	0.048244	4.71E-15	0.176469	0.104339	0.090781
27	rs17637472	17	47461433	G	A	0.393514	0.068629	9.83E-09	0.296541	0.147034	0.043719
28	rs1860509	7	1.39E+08	T	G	-0.31022	0.051367	1.55E-09	-0.06445	0.111553	0.563422
29	rs1887320	20	10965998	A	G	-0.26362	0.048244	4.66E-08	-0.03671	0.104399	0.725108
30	rs2239193	12	1.13E+08	A	G	-0.51507	0.052194	5.81E-23	-0.53602	0.113362	2.27E-06
31	rs2290573	15	75129594	G	A	-0.46924	0.066223	1.39E-12	-0.07856	0.145609	0.589537
32	rs232927	12	1.13E+08	A	G	0.323134	0.051367	3.17E-10	0.32375	0.110422	0.00337
33	rs233722	12	1.13E+08	G	A	0.389463	0.049603	4.13E-15	0.529597	0.106747	7.02E-07
34	rs2398770	7	1.31E+08	T	G	0.284994	0.04815	3.25E-09	0.084642	0.104336	0.417227
35	rs2681492	12	90013089	T	C	-0.68051	0.049981	3.43E-42	-0.10769	0.107808	0.317857
36	rs268263	2	1.65E+08	A	T	-0.57146	0.04885	1.34E-31	0.05449	0.106265	0.60811
37	rs2880099	4	1.56E+08	A	C	-0.26743	0.048797	4.25E-08	0.082864	0.105762	0.433337
38	rs2943810	11	61279799	G	C	-0.41931	0.048255	3.67E-18	-0.19498	0.104271	0.061494
39	rs357305	2	1.65E+08	C	T	-0.26762	0.048416	3.25E-08	-0.02849	0.10465	0.785415
40	rs373894	11	9763094	A	C	0.406373	0.048862	9.11E-17	-0.19071	0.105513	0.070692
41	rs3860432	2	1.65E+08	T	C	-0.33006	0.053727	8.1E-10	0.070174	0.116383	0.546539
42	rs3931703	2	51013453	C	G	0.313462	0.054398	8.31E-09	0.009283	0.118669	0.937646
43	rs438885	2	1.65E+08	T	A	-0.33476	0.050782	4.35E-11	0.144143	0.109394	0.187624
44	rs4693128	4	86730714	C	T	0.298365	0.048338	6.74E-10	0.245279	0.104324	0.01872
45	rs4757380	11	16035345	T	G	0.304343	0.054331	2.13E-08	0.042563	0.118034	0.718399
46	rs4767014	12	1.13E+08	T	C	-0.4155	0.05396	1.37E-14	-0.41963	0.11913	0.000428
47	rs4767366	12	1.16E+08	T	C	-0.33563	0.051836	9.52E-11	-0.09634	0.112903	0.393482
48	rs55651363	9	1.3E+08	C	T	-0.5977	0.108076	3.2E-08	-0.49012	0.234292	0.036451
49	rs55946641	6	25456913	C	T	0.423732	0.067679	3.84E-10	0.191191	0.146357	0.191444
50	rs57625069	3	23438574	A	C	0.419102	0.060008	2.88E-12	-0.57492	0.127495	6.51E-06
51	rs57931766	12	1.16E+08	T	C	-0.27612	0.048688	1.42E-08	0.047305	0.105235	0.65306
52	rs58274947	6	1.27E+08	C	T	0.273472	0.048326	1.53E-08	-0.17658	0.104828	0.09209

53	rs62033408	16	53827962	A	G	0.521087	0.07312	1.03E-12	0.763422	0.158537	1.47E-06
54	rs6483656	11	9771687	C	G	-0.32515	0.053739	1.45E-09	0.095647	0.116363	0.411094
55	rs6489885	12	1.13E+08	A	G	0.276815	0.048874	1.48E-08	0.250284	0.105833	0.018038
56	rs6489979	12	1.12E+08	C	T	0.31316	0.054918	1.18E-08	0.267553	0.118016	0.023388
57	rs6504411	17	46672154	C	T	-0.34896	0.062001	1.82E-08	0.108007	0.134476	0.421881
58	rs671	12	1.12E+08	G	A	-1.29641	0.065532	5.41E-87	-1.69679	0.14402	5.21E-32
59	rs68047333	8	25914085	T	G	0.301588	0.048945	7.21E-10	0.066378	0.106026	0.53128
60	rs7131442	11	16348061	A	T	0.385314	0.053987	9.57E-13	-0.08954	0.116447	0.441914
61	rs74157561	10	1.16E+08	A	G	0.459817	0.074267	5.98E-10	-0.00291	0.160742	0.98556
62	rs74601708	7	995724	T	C	0.337936	0.051583	5.72E-11	0.051024	0.111513	0.647268
63	rs74661587	5	1.22E+08	G	A	-0.41757	0.048607	8.73E-18	-0.09781	0.106001	0.356162
64	rs75642389	12	1.13E+08	C	G	0.445016	0.08031	3.01E-08	0.204914	0.161026	0.203182
65	rs7686601	4	81160632	G	C	-0.36526	0.050084	3.05E-13	0.024542	0.10876	0.821472
66	rs77180047	10	1.05E+08	G	A	-0.55376	0.055676	2.66E-23	-0.19802	0.120199	0.099467
67	rs80111044	4	81168104	A	G	-0.40642	0.053415	2.78E-14	0.07835	0.115331	0.496921
68	rs9687065	5	1.48E+08	A	G	-0.31836	0.055107	7.61E-09	-0.08657	0.119002	0.466942

Abbreviations: SNP, single nucleotide polymorphisms; CHR, chromosome; GWAS, genome-wide association study; KCPS-II, Korean cancer prevention study-II; KoGES, korean genome and epidemiology study; SE, standard error

2. General characteristics for the mendelian randomization analysis

Bidirectional causal association with genetically determined FBS or SBP was assessed based on mendelian randomization (MR) analysis using by 2-stage least squares (2SLS) regression model.

Table 3 displays the general characteristics of the study population. The mean ages of the participants involved in the MR analysis was 52.0 years (SD=8.9 years), and there were 47.4% men. At baseline, more than 10% developed T2D and a hypertension prevalence of 19% was identified. The mean of the main phenotypes (FBS levels and SBP measurements) was 92.7 mg/dL (SD=23.1) and 117.4 mmHg (SD=18.1), respectively. When the general characteristics were stratified by men and women, women's age (52.4 year, SD=9.0 years for men; 51.6 year, SD=8.7 years for women; p<.0001), body mass index (BMI) at the first visit (24.3 kg/m2, SD=2.9 for men; 24.9 kg/m2, SD=3.3 for women; p<.0001), and the FBS level at the first visit (95.1 mg/dL, SD=25.0 for men; 90.6 mg/dL, SD=21.2 for women; p<0.0001) were statistically significant.

The significant bidirectional correlations are shown in Figures 6 and 7 (β=0.0102, p=0.0050 for $wGRS_{FBS}$→SBP; β=0.0072, p<.0001 for $wGRS_{SBP}$→FBS).

Table 3. General characteristics of the study population in the mendelian randomization analysis

		Total (N=8,510)	Men (N=4,031)	Women (N=4,479)	
Subject		Mean ± SD	Mean ± SD	Mean ± SD	p-value*
Age, year		52.02 ± 8.85	51.59 ± 8.71	52.40 ± 8.96	<.0001
Body mass index, kg/m^2		24.59 ± 3.14	24.25 ± 2.94	24.91 ± 3.27	<.0001
Fasting blood sugar, mg/dL		92.74 ± 23.14	95.09 ± 24.95	90.61 ± 21.15	<.0001
Systolic blood pressure, mmHg		117.44 ± 18.06	117.51 ± 16.57	117.38 ± 19.30	0.7273
Diastolic blood pressure, mmHg		75.00 ± 11.29	75.54 ± 10.82	74.03 ± 11.58	<.0001
Total cholesterol, mg/dL		199.02 ± 36.78	198.63 ± 36.64	199.36 ± 36.91	0.3590
Triglyceride, mg/dL		152.43 ± 108.81	170.23 ± 124.41	136.42 ± 89.60	<.0001
		N (%)	N (%)	N (%)	
Smoking status	Former	243 (2.86)	186 (4.61)	57 (1.27)	<.0001
	Current	1903 (22.36)	1802 (44.70)	101 (2.25)	<.0001
Alcohol drinking	Yes	4543 (53.38)	3265 (81.00)	1278 (28.53)	<.0001
Exercise	Yes	8167 (95.97)	3879 (96.23)	4288 (95.74)	0.2477
$wGRS_{91snp\ for\ FBS}$	Q1	2128 (25.01)	1030 (25.55)	1098 (24.51)	0.2698
	Q2	2127 (24.99)	1011 (25.08)	1116 (24.92)	0.8612
	Q3	2128 (25.01)	971 (24.09)	1157 (25.83)	0.0637
	Q4	2127 (24.99)	1019 (25.28)	1108 (24.74)	0.5646
$wGRS_{91snp\ for\ SBP}$	Q1	2128 (25.01)	1011 (25.08)	1117 (24.94)	0.8799
	Q2	2127 (24.99)	1001 (24.86)	1125 (25.12)	0.7822
	Q3	2126 (24.98)	1004 (24.91)	1122 (25.05)	0.8788
	Q4	2129 (25.02)	1014 (25.16)	1115 (24.89)	0.7813
Antidiabetic treatment		1010 (11.93)	480 (11.98)	530 (11.89)	0.8985
Antihypertensives treatment		972 (11.42)	379 (9.40)	593 (13.24)	<.0001
Type 2 diabetes prevalence		892 (10.48)	478 (11.86)	414 (9.24)	<.0001
Hypertension prevalence		1616 (18.99)	699 (17.34)	917 (20.47)	0.0002

Abbreviations: SD, standard deviation; N, number; GRS, genetic risk score; Q quatiles
*p-value for differences between FBS trajectory groups based on T-test or chi-square test

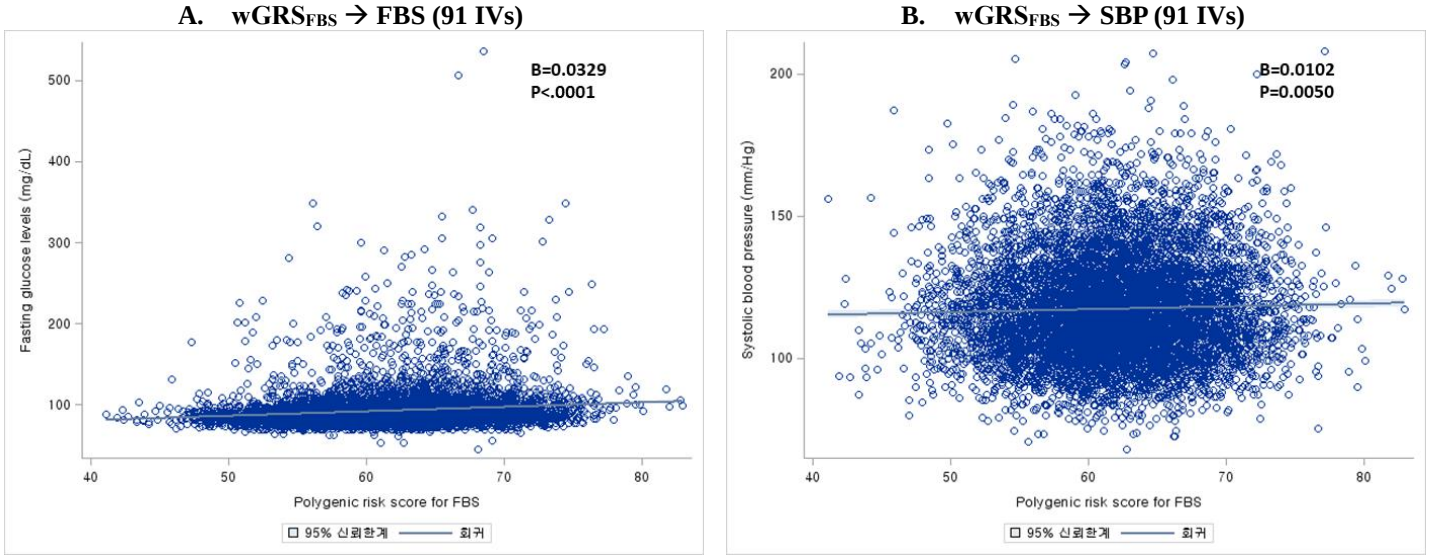
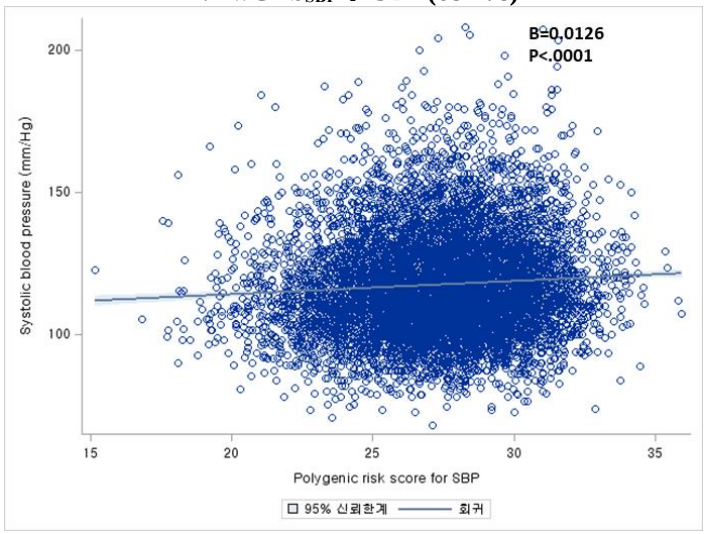


Figure 6. Associations between weighted genetic risk score for fasting blood sugar levels and each phenotype (FBS and SBP) levels based on linear regression adjusted age and sex

Abbreviations: FBS, fasting blood sugar; SBP, systolic blood pressure; GRS, genetic risk score; IV, instrument variant

A. $wGRS_{SBP}$ → SBP (68 IVs)



B. $wGRS_{SBP}$ → FBS (68 IVs)

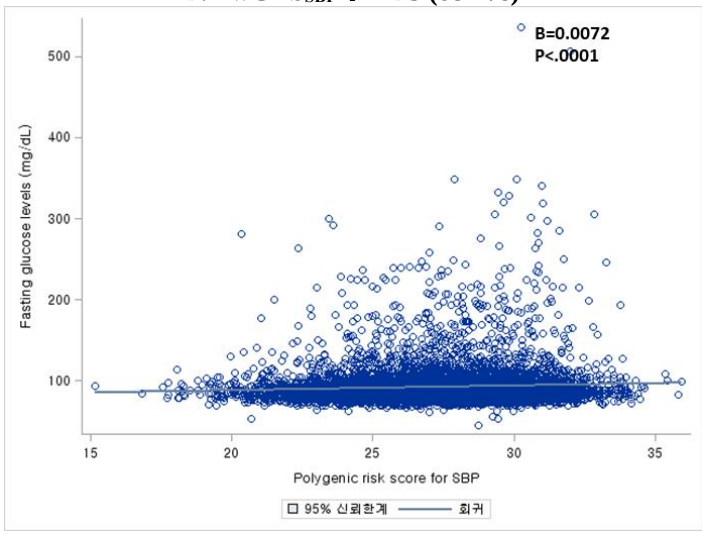


Figure 7. Associations between weighted genetic risk score systolic blood pressure levels and each phenotype (FBS and SBP) levels based on linear regression adjusted age and sex

Abbreviations: FBS, fasting blood sugar; SBP, systolic blood pressure; GRS, genetic risk score; IV, instrument variant

3. Assessing the possibility of a bidirectional-causality based on the 2-stage least squares (2SLS) regression model

3-1. A bidirectional-causality of FBS and SBP

The possibility of bidirectional-causality assessed based on 2SLS regression model though the hypothesis; genetically determined high FBS would be significantly associated with an increase in SBP risk without the influence of confounders and vice versa. Bidirectional MR analysis was implemented based on the frame shown in **Figure 8**. In this frame, wGRS for for the two main phenotypes (FBS and SBP) was used as genetic instrumented variants. Weak instrument test and endogenous test were performed to wGRS of two main phenotypes (FBS and SBP). Then, the results were assessed to determine whether wGRS appropriately satisfied the 2SLS regression model as an instrumented variant or not using F-statistic and p values for the endogenous test.

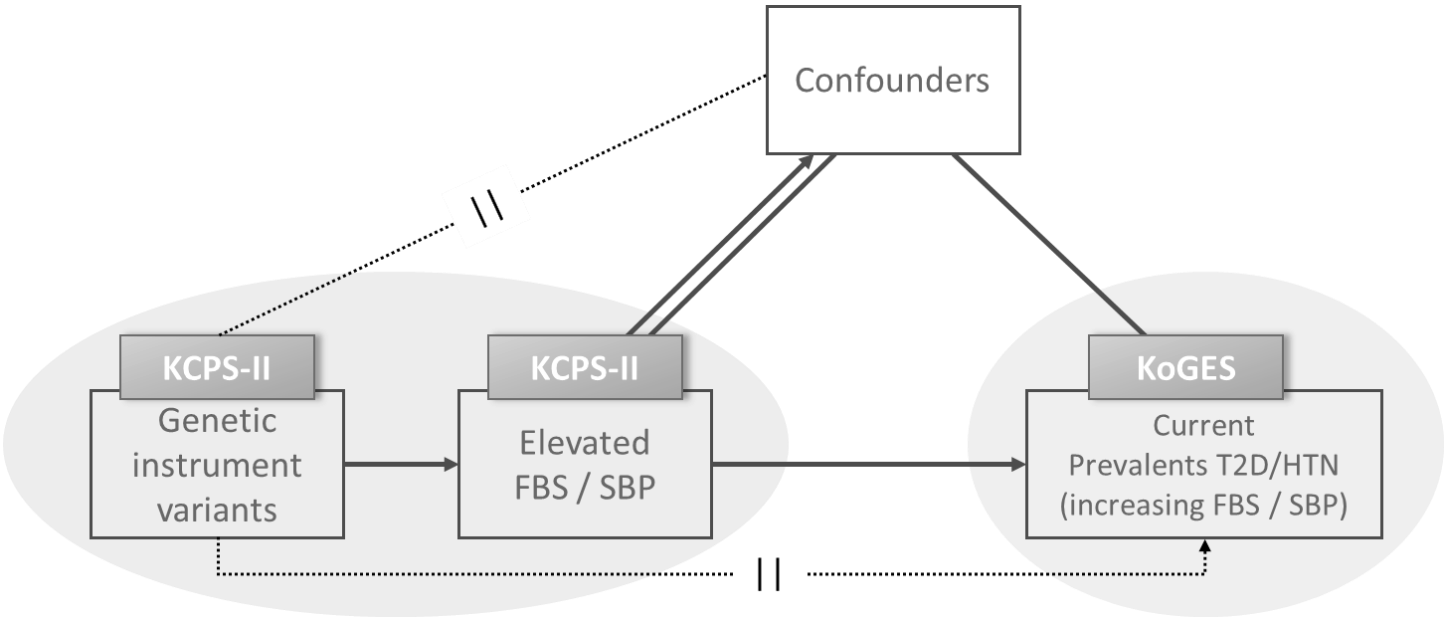


Figure 8. A schematic frame for 2 sample MR analysis

A schematic figure of the bidirectional association between FBS and SBP inferred from Mendelian randomization. Genetically determined high FBS would be significantly associated with an increase in SBP risk without the influence of confounders and vice versa.

The results of the 2SLS regression model demonstrated a significant bidirectional causality (**Figure 9 and 10**). In the FBS to SBP direction, an increase of 1.63 mm/Hg in the SBP was detected when FBS was 10 mg/dL or higher. In contrast, an increase of 11.39 mg/dL in the FBS was detected when the SBP was 10 mm/Hg or higher in the SBP→FBS direction. When the analyses were expanded to include other metabolic components, FBS to diastolic blood pressure (DBP) direction was significant (β=0.87, p=0.024 per FBS 10 mg/dL). Moreover, hemoglobin A1c (HbA1c) and triglyceride (TG) had significant result related to SBP (β=0.16, p=0.037 per SBP 10 mm/Hg for HbA1c; β=23.48, p=0.016 per SBP 10 mm/Hg for TG).

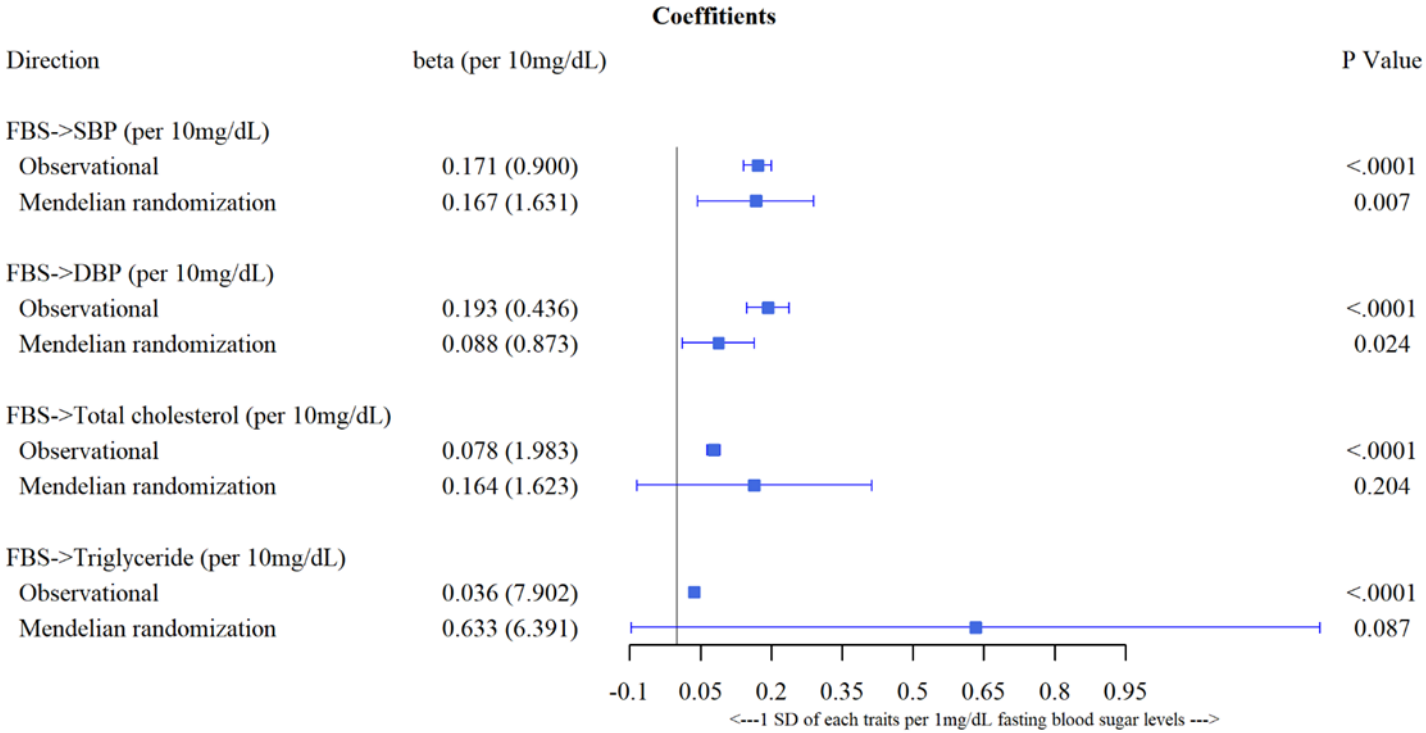


Figure 9. The effects of FBS on metabolic components from Observational and Mendelian randomization analyses testing

The adjusted effect size from multiple linear regression model adjusted for age and sex, with 1 SD of cardiometabolic traits per 10 mg/dL higher FBS. The SD of the corresponding analysis is shown in parentheses.

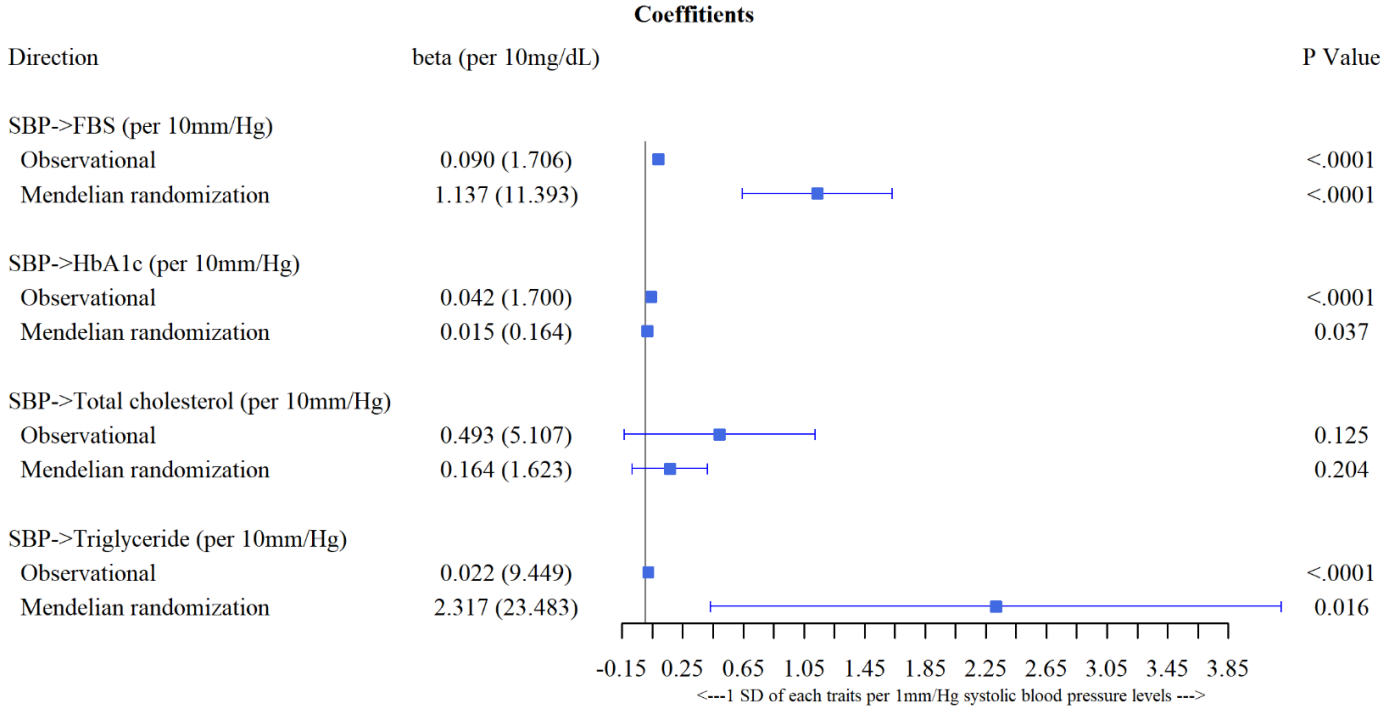


Figure 10. The effects of SBP on metabolic components from Observational and Mendelian randomization analyses testing

The adjusted effect size from multiple linear regression model adjusted for age and sex, with 1 SD of cardiometabolic traits per 10 mm/Hg higher SBP. The SD of the corresponding analysis is shown in parentheses.

3-2. A bidirectional causality in the prevalence of T2D / hypertension (at baseline)

Not only was the 2SLS regression model applied to the two continuous phenotypes (FBS and SBP), an additional model with binary phenotypes (the presence or absence of T2D or hypertension) at baseline was also implemented based on the framework shown in **Figure 8**.

In the FBS to T2D direction, significant odds ratio (OR) of 3.12 for T2D ($p<.0001$) was detected as the FBS level increased to 10 mg/dL, based on the endogenous test ($p=0.0101$). In the FBS to hypertension direction, the OR for hypertension for an increase in the FBS level to 10 mg/dL was not significant (OR=1.14, $p=0.143$) (**Table 4**).

Also, there was significant 2.72 OR for hypertension ($p<.0001$) as per increasing SBP 10 mm/Hg for SBP→Hypertension direction, but this result had not significant p-value based on endogenous test ($p=0.2829$). On the other side, for SBP→T2D direction, borderline significant causal association passed endogenous test ($p=0.0195$) was shown; 1.72 OR for T2D ($p=0.0610$) as per increasing SBP 10 mm/Hg. a strong significance was noted for for T2D in men (OR=3.39, $p<.0001$) as per increasing SBP 10 mm/Hg was identified in men (**Table 5**).

Table 4. Odds ratios of FBS on prevalents of T2D / hypertension from Observational and Mendelian randomization analyses testing

			Observational multivariable regression analysis X-Y				Mendelian randomization analysis					
			OR	**LC**	**UC**	**p**	**F-statistic G-X**	**P for endogenous test†**	**OR**	**LC**	**UC**	**p**
FBS→T2D (per 10 mg/dL)	wGRS for FBS (N=91)	**Total***	3.908	3.604	4.238	<.0001	157.39	0.0101	3.122	2.479	3.932	<.0001
		Men	3.648	2.324	4.074	<.0001	109.25	0.3836	3.123	2.288	4.263	<.0001
		Women	4.215	3.745	4.743	<.0001	51.65	0.0050	3.086	2.188	4.352	<.0001
FBS→HTN (per 10 mg/dL)	wGRS for FBS (N=91)	**Total***	1.115	1.089	1.142	<.0001	157.39	0.9184	1.144	0.956	1.370	0.143
		Men	1.102	1.067	1.138	<.0001	109.25	0.7338	1.142	0.879	1.483	0.321
		Women	1.128	1.089	1.169	<.0001	51.65	0.6866	1.131	0.887	1.451	0.334

Abbreviations: FBS, fasting blood sugar; GRS, genetic risk score; OR, odds ratio; LC, lower confidence interval; UC, upper confidence interval; G, genetic variant; X, dependent variable

*Adjusted for age and sex (Adjusted for age only when model was stratified into men and women)

†Tests of endogeneity is Wu-Hausman test, Ho: variables are exogenous

Table 5. Odds ratios of SBP on prevalents of T2D / hypertension from Observational and Mendelian randomization analyses testing

			Observational multivariable regression analysis X-Y				Mendelian randomization analysis					
			OR	**LC**	**UC**	**p**	**F-statistic G-X**	**P for endogenous test†**	**OR**	**LC**	**UC**	**p**
SBP→HTN (per 10 mm/Hg)	wGRS for SBP (N=68)	**Total***	2.271	2.163	2.385	<.0001	40.64	0.2829	2.721	1.748	4.237	<.0001
		Men	2.286	2.124	2.460	<.0001	39.54	0.1007	4.621	2.438	8.760	<.0001
		Women	2.252	2.110	2.404	<.0001	10.16	0.9834	1.740	0.942	3.212	<.0001
SBP→T2D (per 10 mm/Hg)	wGRS for SBP (N=68)	**Total***	**1.158**	**1.113**	**1.204**	**<.0001**	**40.64**	**0.0195**	**1.715**	**0.975**	**3.014**	**0.061**
		Men	**1.111**	**1.049**	**1.177**	**<.0001**	**39.54**	**0.0068**	**3.392**	**1.593**	**7.218**	**0.002**
		Women	1.190	1.128	1.256	<.0001	10.16	0.6062	0.790	0.341	1.827	0.581

Abbreviations: SBP, systolic blood pressure; GRS, genetic risk score; OR, odds ratio; LC, lower confidence interval; UC, upper confidence interval; G, genetic variant; X, dependent variable

*Adjusted for age and sex (adjusted for age only when model was stratified into men and women)

†Tests of endogeneity is Wu-Hausman test, Ho: variables are exogenous

3-3. The Sensitivity of the mendelian randomization analysis

The sensitivity of the MR analysis was assessed to test for the evidence of horizontal pleiotropy. All analyses including the IVW method, median-based methods (simple and weighted), and the MR-Egger method, were performed in compliance with the 3 IV assumptions and InSIDE assumptions. A bidirectional sensitivity MR analysis to determine whether a bidirectional-causal association existed between genetic instrument variants and two phenotypes (FBS or SBP) was conducted. The results of this bidirectional MR analysis for both of phenotype (FBS or SBP) were obtained from four methods including IVW method, median-based methods (simple and weighted) and MR-egger method.

Genetically, a 1-mg/dL increase in the FBS level was associated with a 0.19-mmHg increase in the SBP measurement ($p<.0001$) when the IVW method was used for the MR analysis (**Table 6**). This result was statistically significant after Bonferroni multiple testing correction [$p<0.0005$ (=0.05/91)]. For the MR-Egger method, a 1-mg/dL genetic increase in the FBS level was associated with a 0.20-mmHg increase in the SBP measurement ($p<.0001$), but the MR-Egger intercept was not significant (intercept=-0.01, $p=0.823$). Furthermore, the results were significant for both of the median methods (simple and weighted median).

In the direction from SBP to FBS, a 1-mm/Hg genetic increase in the SBP measurement was associated with a 0.57-mg/dL increase in the FBS level (p<.0001) using the IVW method (**Table 6**). This causal relationship remained significant after the Bonferroni multiple testing correction [p<0.0007 (=0.05/68)]. The results evaluated using the two median-based methods (simple and weighted) were also significant. For the MR-Egger method, genetically, a 1- mmHg increase in the FBS level was associated with a 1.08-mg/dL increase in the SBP measurement (p<.0001). The MR-Egger intercept was also significant (intercept=-0.23, p=0.001). Therefore, in the SBP to FBS direction, a significant horizontal pleiotropy was confirmed.

In both directions, the most of outstanding outlier was the rs671 polymorphism. After the omission of the rs671 polymorphism, a non-significant MR-Egger intercept (intercept=-0.10, p=0.196) was shown (**Table 7**) in. the SBP to FBS direction. When two additional outliers were omitted, the MR-Egger intercept was not significant (intercept=0.05, p=0.465) (**Table 7**).

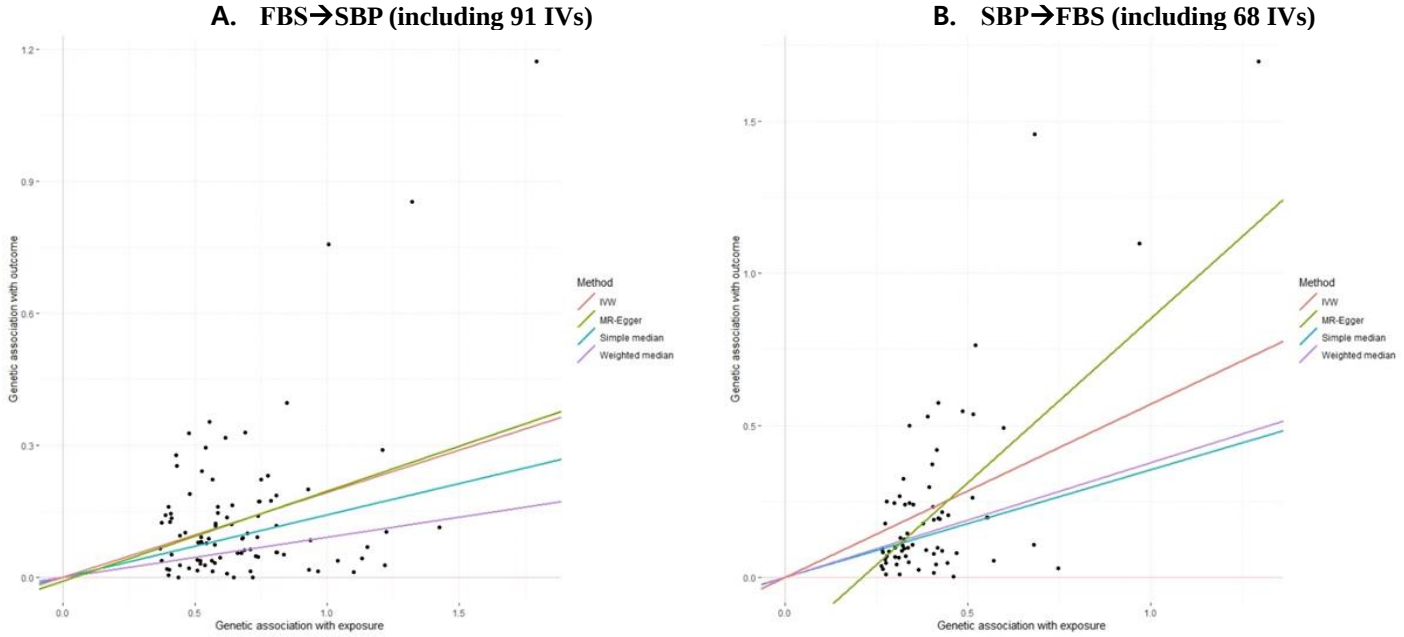


Figure 11. Scatter plots for associations between genetic instrument variants and each phenotype based on mendelian randomization analysis

Abbreviations: FBS, fasting blood sugar; SBP, systolic blood pressure; IV, instrument variant; IVW, inverse variance weighted; MR, mendelian randomization

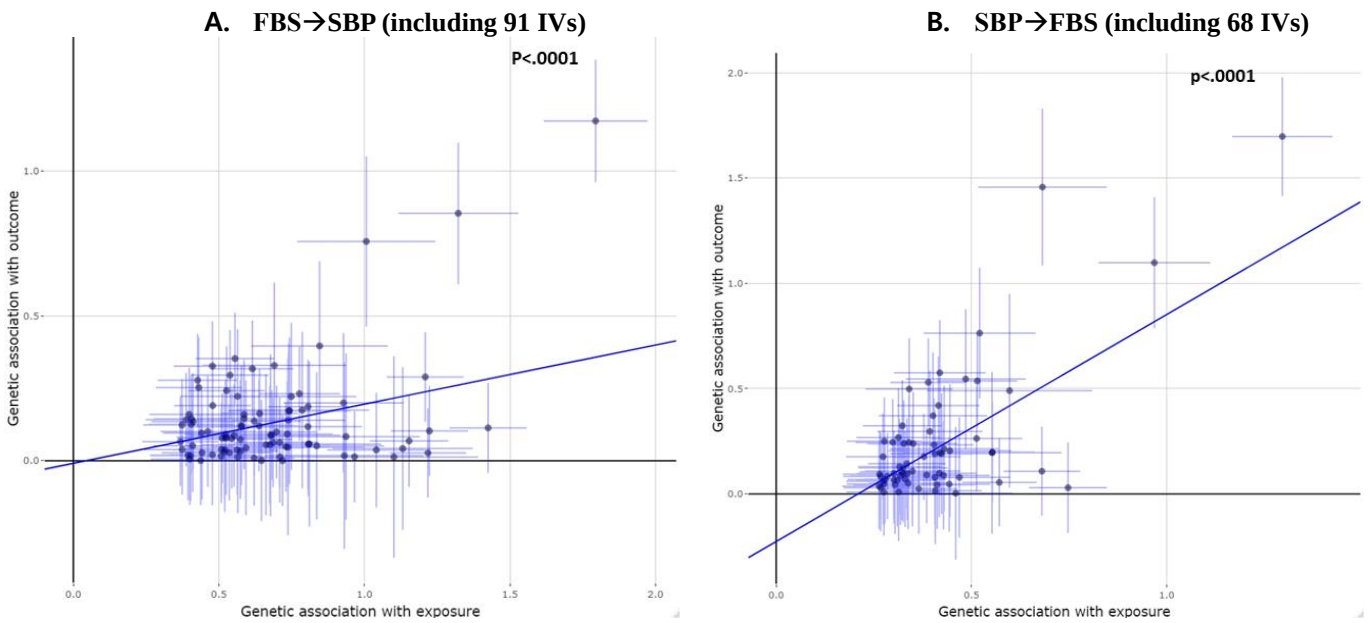
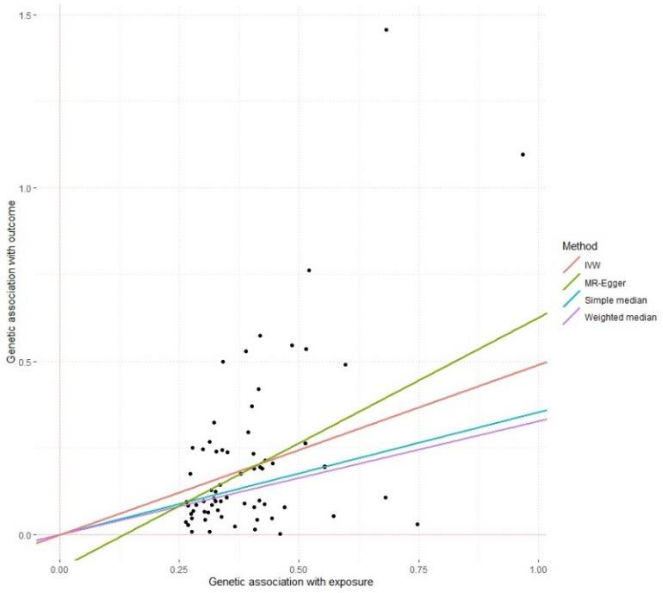


Figure 12. MR-egger scatter plots for weighted polygenic risk score based on mendelian randomization analysis

Abbreviations: FBS, fasting blood sugar; SBP, systolic blood pressure; IV, instrument variant; MR, mendelian randomization

A. SBP→FBS (including 67 IVs without rs671)



B. SBP→FBS (including 65 IVs without 3 outliers)

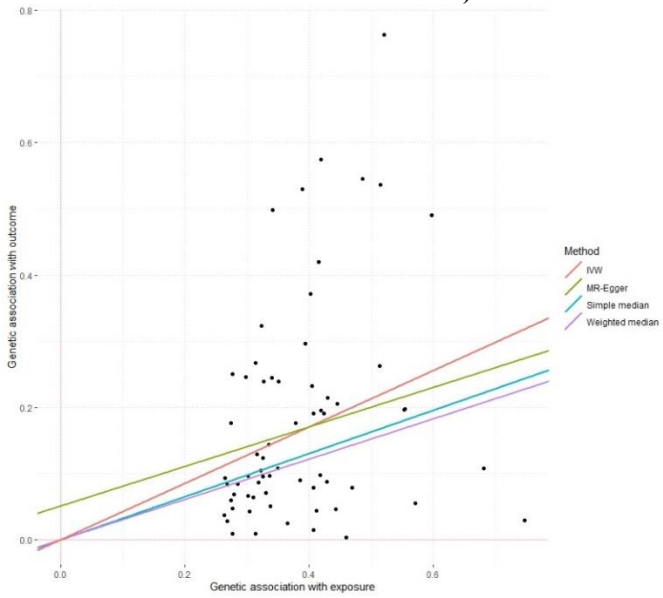


Figure 13. Scatter plots for associations between genetic instrument variants for SBP and FBS based on mendelian randomization analysis without outliers

Table 6. bidirectional associations between FBS and SBP based on Mendelian Randomization analysis using Genetic Instrument Variables

	FBS → SBP (No. of genetic IVs=91)			**SBP → FBS** (No. of genetic IVs=68)		
	Effect size	S.E.	p-value	Effect size	S.E.	p-value
2SLS[*]	0.167	0.063	0.0070	1.137[†]	0.252	<.0001
IVW	0.192	0.021	<.0001	0.569	0.060	<.0001
Simple median	0.142	0.024	<.0001	0.354	0.061	<.0001
Weighted median	0.091	0.023	<.0001	0.376	0.062	<.0001
MR-egger	0.204	0.057	<.0001	1.078	0.157	<.0001
(Intercept)	-0.009	0.039	0.8230	-0.226	0.065	0.0010

Abbreviations: FBS, fasting blood sugar; SBP, systolic blood pressure; IV, instrument variant; IVW, inverse variance weighted; MR, mendelian randomization

[*]2-stage least squares (2SLS) regression model using weighted genic risk score (wGRS) for FBS or SBP

[†]There was a significant p-value for endogenous test (Wu-Hausman test, Ho: variables are exogenous)

Table 7. Causal associations on SBP to FBS from sensitivity mendelian Randomization analysis using Genetic Instrument Variables without outliers

	SBP → FBS (No. of genetic IVs=67) Without rs671			(No. of genetic IVs=65) Without 3 outliers		
	Effect size	S.E.	p-value	Effect size	S.E.	p-value
2SLS*	1.623†	0.269	<.0001	1.159†	0.279	<.0001
IVW	0.490	0.056	<.0001	0.426	0.047	<.0001
Simple median	0.353	0.061	<.0001	0.326	0.061	<.0001
Weighted median	0.327	0.056	<.0001	0.305	0.059	<.0001
MR-egger	0.722	0.188	<.0001	0.298	0.181	<.0001
(Intercept)	-0.097	0.075	0.1960	0.051	0.070	0.4650

Abbreviations: FBS, fasting blood sugar; SBP, systolic blood pressure; IV, instrument variant; IVW, inverse variance weighted; MR, mendelian randomization

*2-stage least squares (2SLS) regression model using weighted genic risk score (wGRS) for SBP

†There was a significant p-value for endogenous test (Wu-Hausman test, Ho: variables are exogenous)

Table 8. Summary for the results from mendelian Randomization analysis

	Significance for 2SLS regression model	Significance for endogenous test†	Significance for pleiotropy under MR-egger
FBS→SBP	O	X	X
FBS→HTN	△	X	-
SBP→FBS	O	O	O*
SBP→T2D	O	O	-

Abbreviations: FBS, fasting blood sugar; SBP, systolic blood pressure; 2SLS, 2-stage least squares

*Pleiotropy was disappeared after omitted rs671 (top outlier)

†Tests of endogeneity is Wu-Hausman test, Ho: variables are exogenous

PART II. Association analyses for future T2D or hypertension

1. General characteristics of the healthy general population

Table 9 displays the general characteristics of the healthy general population without T2D, hypertension, or stroke at baseline. The mean age of the participants was 50.7 years (SD=8.6 years), and there were 47.5% men. During the follow-up, 68.9% individuals developed hypertension, and 51.3% individuals developed T2D. Moreover, the FBS and SBP measurements at the 8th visit was higher than at the 1st visit. When the general characteristics were stratified by men and women, there was no difference in the mean age (mean=51.7 years and SD=8.6 years for men; mean=50.7 years and SD=8.6 years for women; p=8367). The BMI at the first visit was higher among women (23.96 kg/m2, SD=2.9 kg/m2 for men; 24.40 kg/m2, SD=3.1 kg/m2 for women; p<.0001). In contrast, the FBS level (89.1 mg/dL, SD=9.2 for men; 86.1 mg/dL, SD=7.9 for women; p<.0001), SBP measurement (113.7 mm/Hg, SD=13.6 for men; 111.4 mm/Hg, SD=14.8 for women; p<.0001), DBP (74.4 mm/Hg, SD=9.4 for men; 70.9 mm/Hg, SD=9.5 for women; p<.0001), and TG (161.6 mg/dL, SD=118.7 for men; 123.9mg/dL, SD=78.2 for women; p<.0001) at the first visit, men had much higher values than women (**Table 9**).

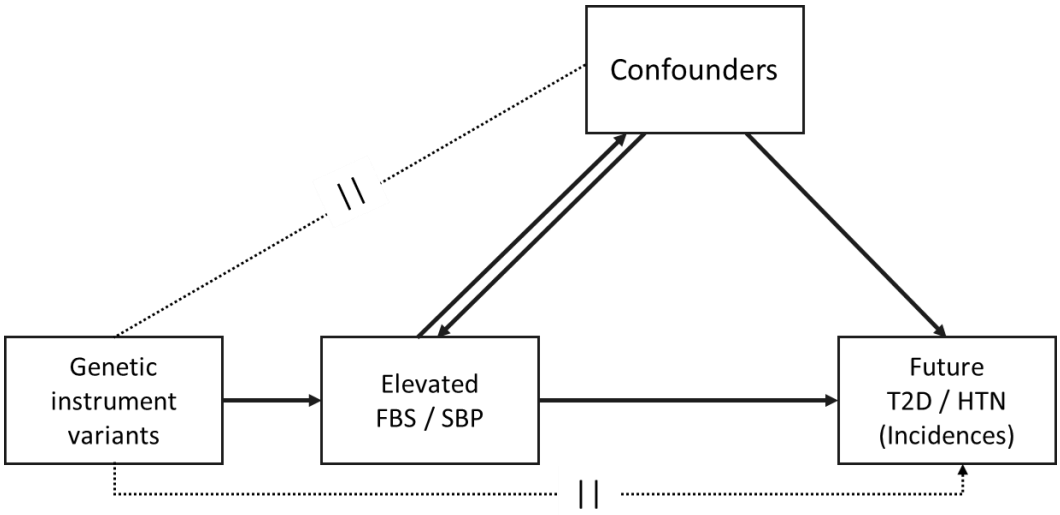


Figure 14. A schematic frame for the association analysis related to genetic instrument variants and future T2D or hypertension

A schematic figure of the association between PRS for FBS / SBP based on time-independent genetic variants and future incidents of T2D or hypertension in Korean middle-aged healthy population. All heathy participants had weighted genetic risk score based on genetic instrument variants passed MR analysis.

Table 9. General characteristics of the healthy general population

		Total (N=6,278)	Men (N=2,979)	Women (N=3,299)	
Subject		Mean ± SD	Mean ± SD	Mean ± SD	p-value*
Age, year		50.69 ± 8.58	50.66 ± 8.55	50.71 ± 8.61	0.8367
Body mass index, kg/m^2	1st Visit	24.19 ± 3.01	23.96 ± 2.87	24.40 ± 3.11	<.0001
	8th Visit	24.52 ± 3.23	24.56 ± 3.25	24.49 ± 3.22	0.4403
Fasting blood sugar, mg/dL	1st Visit	87.53 ± 8.69	89.13 ± 9.24	86.09 ± 7.88	<.0001
	8th Visit	102.16 ± 26.53	102.07 ± 26.67	102.24 ± 26.42	0.8425
Systolic blood pressure, mmHg	1st Visit	112.49 ± 14.29	113.68 ± 13.57	111.42 ± 14.83	<.0001
	8th Visit	119.29 ± 15.15	119.31 ± 15.07	119.29 ± 15.23	0.9661
Diastolic blood pressure, mmHg	1st Visit	72.59 ± 9.61	74.41 ± 9.39	70.94 ± 9.51	<.0001
	8th Visit	72.76 ± 9.36	73.02 ± 9.33	72.52 ± 9.39	0.0847
Total cholesterol, mg/dL		196.10 ± 35.49	196.90 ± 35.41	195.38 ± 35.55	0.0894
Triglyceride, mg/dL		141.79 ± 101.28	161.61 ± 118.71	123.90 ± 78.24	<.0001
		N (%)	N (%)	N (%)	
Smoking status	Former	183 (2.91)	142 (4.77)	41 (1.24)	<.0001
	Current	1447 (23.05)	1373 (46.09)	74 (2.24)	<.0001
Alcohol drinking	Yes	3384 (53.90)	2391 (80.26)	993 (30.10)	<.0001
Exercise	Yes	6026 (95.99)	2869 (96.31)	3158 (95.70)	0.2175
$wGRS_{91snps\ for\ FBS}$	Q1	1570 (25.01)	764 (25.65)	806 (24.43)	0.2672
	Q2	1569 (24.99)	753 (25.28)	816 (24. 37)	0.6203
	Q3	1569 (24.99)	714 (23.97)	855 (25.58)	0.0742
	Q4	1570 (25.01)	748 (25.11)	822 (24.92)	0.8604
$wGRS_{68snps\ for\ SBP}$	Q1	1570 (25.01)	786 (26.38)	784 (23.76)	0.0167
	Q2	1569 (24.99)	723 (24.27)	846 (25.64)	0.2092
	Q3	1569 (24.99)	727 (24.40)	842 (25.52)	0.3066
	Q4	1570 (25.01)	743 (24.94)	827 (25.07)	0.9077
Type 2 diabetes incident		3220 (51.29)	1519 (50.99)	1701 (51.56)	0.6514
Hypertension incident		4323 (68.86)	2032 (68.21)	2291 (69.45)	0.2915

Abbreviations: SD, standard deviation; N, number; GRS, genetic risk score
*p-value for differences between FBS trajectory groups based on T-test or chi-square test

2. A bidirectional association between the wGRS and future T2D or hypertension

2-1. The wGRS for FBS and the future risk of hypertension

Table 10 shows the association between the wGRS quartile groups for FBS and the future risk of hypertension in the healthy general population without T2D, hypertension, or stroke at baseline. In the Cox proportional hazard model, adjusted for age, sex, BMI, TG, smoking behavior, alcohol consumption, exercise and the use of antihypertensives medications, the second to the fourth PRS quartile groups did not demonstrate a significant risk of subsequent hypertension compared to the first PRS quartile group (HR: 0.95, 95% CI: 0.87-1.03 for quartile 2; HR: 0.95, 95% CI: 0.87-1.04 for quartile 3; HR: 0.96, 95% CI: 0.88-1.05 for quartile 4) (**Table 10**).

2-2. The wGRS for SBP and the future risk of T2D

For wGRS quartile groups for SBP and with future T2D, 2^{nd} to 4^{th} PRS quartile groups had not significant risk of subsequence T2D compared to 1^{st} PRS quartile group (HR: 1.02, 95% CI: 0.93-1.13 for quartile 2; HR: 1.03, 95% CI: 0.93-1.14 for quartile 3; HR: 0.94, 95% CI: 0.85-1.04 for quartile 4) (**Table 11**).

Table 10. Association with Hypertension incident according to quartiles for wGRS of FBS on cox proportional-hazards model

	No. of Persons	No. of HTN	Percentage of HTN Cases	HTN		
				Model 1[a]	Model 2[b]	Model 2[c]
				HR (95% CI)	HR (95% CI)	HR (95% CI)
Q1	1569	1097	69.92	1.0	1.0	1.0
Q2	1570	1083	68.98	0.97 (0.89-1.05)	0.97 (0.89-1.05)	0.95 (0.87-1.03)
Q3	1570	1074	68.41	0.96 (0.87-1.05)	0.96 (0.88-1.05)	0.95 (0.87-1.04)
Q4	1569	1069	68.13	0.96 (0.88-1.04)	0.96 (0.88-1.04)	0.96 (0.88-1.05)
P for trend				0.3407	0.3372	0.4641
-2 LOG L				71245.940	71237.933	70284.265

Abbreviations: HR, hazard ratio; HTN, hypertension; Q quatiles

[a] adjusted for age and sex

[b] adjusted for age, sex, BMI, smoking behavior, alcohol drink and exercise

[c] adjusted for age, sex, BMI, smoking behavior, alcohol drink, exercise, antidiabetic medications and PRS quartiles for SBP

Table 11. Association with type 2 diabetes incident according to quartiles for wGRS of SBP based on cox proportional-hazards model

	No. of Persons	No. of T2D	Percentage of T2D cases	T2D Model 1[a] HR (95% CI)	T2D Model 2[b] HR (95% CI)	Model 2[c] HR (95% CI)
Q1	**1568**	804	51.28	**1.0**	**1.0**	**1.0**
Q2	**1570**	834	53.12	1.02 (0.93-1.12)	1.02 (0.93-1.13)	1.02 (0.93-1.13)
Q3	**1571**	816	51.94	1.02 (0.92-1.12)	1.03 (0.93-1.13)	1.03 (0.93-1.14)
Q4	**1569**	766	48.82	0.91 (0.83-1.01)	0.92 (0.83-1.02)	0.94 (0.85-1.04)
P for trend				**0.0869**	**0.1343**	**0.2699**
-2 LOG L				53730.318	53718.939	52248.956

Abbreviations: T2D, type 2 diabetes; HR, hazard ratio; Q quatiles

[a] adjusted for age and sex

[b] adjusted for age, sex, BMI, smoking behavior, alcohol drink, exercise and antihypertensive medications

[c] adjusted for age, sex, BMI, smoking behavior, alcohol drink, exercise, antihypertensive medications and PRS quartiles for FBS

3. A bidirectional association between changes during follow-ups and the future risk of T2D or hypertension

3-1. Classification of the distinct trajectories for FBS and SBP

Time-varying changes of FBS or SBP were assessed based on trajectory analyses, and later linked to the incidence of T2D or hypertension in the healthy general population. For the trajectory analyses, the mathematical framework as shown in Figure 12 was applied. When the trajectory analysis was performed with 6,278 subjects, two clearly distinct patterns were identified through the best model for the two phenotypes of interest (FBS or SBP).

The two classified FBS patterns were very different . The controlled group's FBS level remained steady during the follow-up period. The of uncontrolled group’s FBS level showed a reverse U-shape (**Figure 13**).

For SBP, two distinct patterns were also identified. The uncontrolled group’s SBP increased rapidly within the 4-year follow-up time and then, rapidly decreased to an endpoint at the last follow-up (**Figure 14**).

3-2. General characteristics of the distinct trajectories for FBS and SBP

For two FBS patterns (**Figure 16**, no differences between FBS trajectories for age, gender, BMI, FBS, SBP, DBP, total cholesterol and TG at baseline (**Table 12**). And also, differences between FBS trajectories for behavior factors on life-style (smoking status, alcohol drinking, regular exercise) were not shown (**Table 12**).

However, in the uncontrolled group, a larger percentage of participants reported talking antidiabetic drugs (33.9% for the controlled group, 89.3% for the uncontrolled group; $p<0.0001$) or antihypertensive drugs (63.9% for the controlled group, 94.4% for the uncontrolled group; $p<0.0001$) medication during the follow-up. The uncontrolled group demonstrated a higher incidence of T2D (49.3% for the controlled group, 100.0% for the uncontrolled group; $p<0.0001$) and hypertension (67.7% for the controlled group, 95.6% for the uncontrolled group; $p<.0001$) during the follow-up period (**Table 12**).

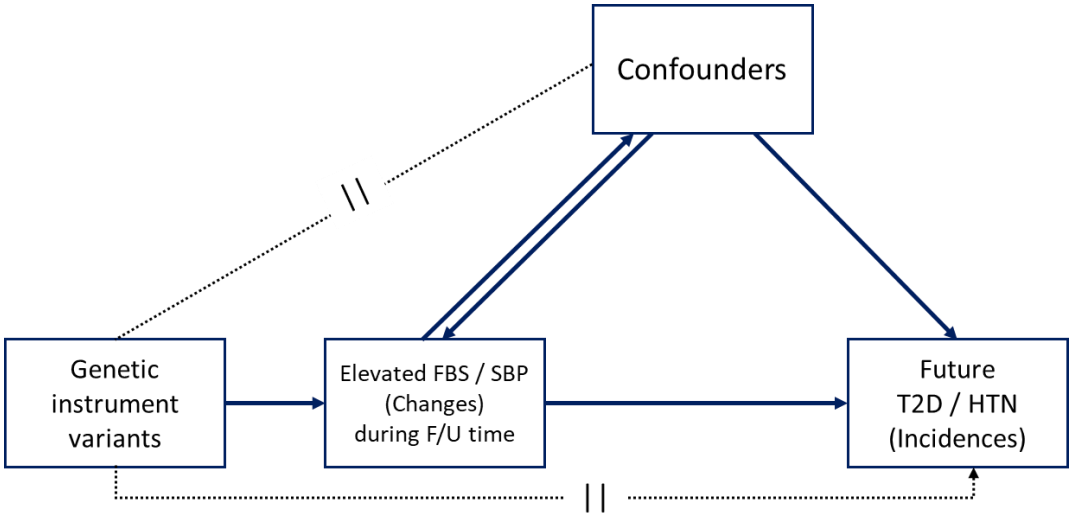


Figure 15. A schematic frame for the association analysis using by trajectory medeling

A schematic figure of the association between time-varying trajectories for genetically determined FBS or SBP and subsequence incident of T2D or hypertension based on trajectory analysis. All heathy participants had weighted genetic risk score based on genetic instrument variants passed MR analysis.

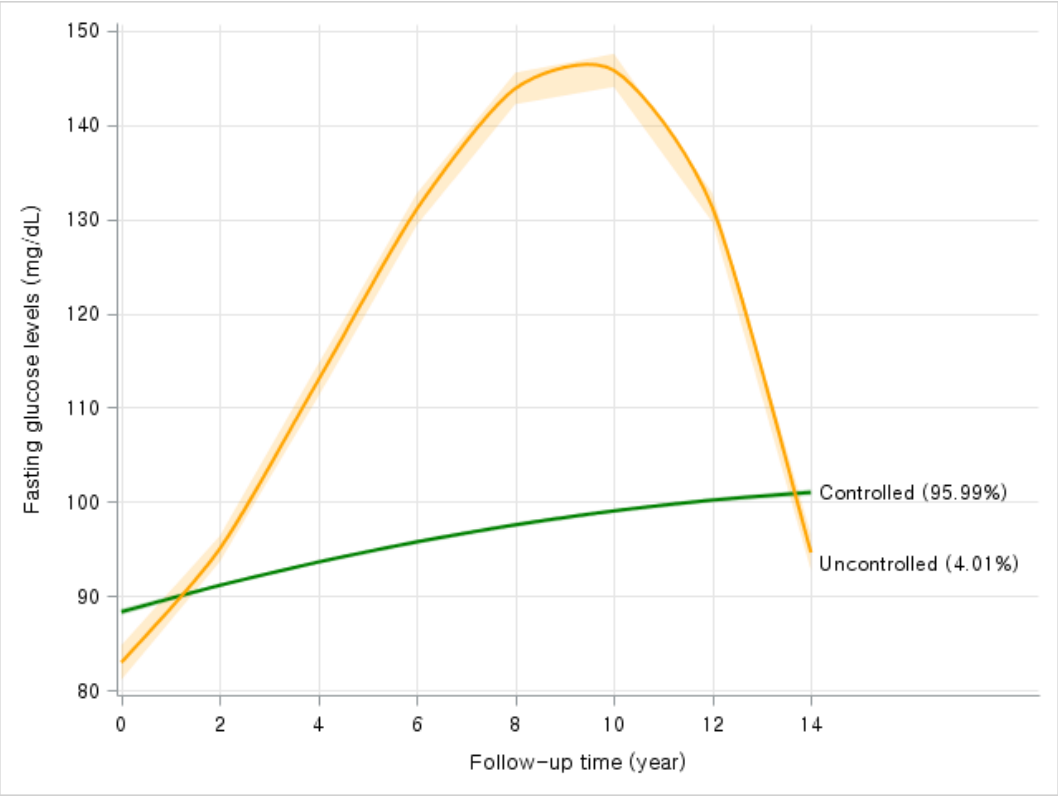


Figure 16. Trajectories of fasting glucose levels in healthy general population

Table 12. General characteristics of the FBS trajectory groups

		Controlled (N=6,026)	Uncontrolled (N=252)	
Subject		Mean ± SD	Mean ± SD	p-value*
Age, year		50.69 ± 8.59	50.63 ± 8.43	0.9192
Body mass index, kg/m^2	**1st Visit**	24.20 ± 3.01	24.02 ± 2.96	0.3648
	8th Visit	24.53 ± 3.23	24.41 ± 3.34	0.5907
Fasting blood sugar, mg/dL	**1st Visit**	87.55 ± 8.68	87.09 ± 8.74	0.4125
	8th Visit	102.09 ± 26.10	103.42 ± 32.91	0.5496
Systolic blood pressure, mmHg	**1st Visit**	112.55 ± 14.29	111.02 ± 14.27	0.0953
	8th Visit	119.31 ± 15.16	119.19 ± 15.09	0.9068
Diastolic blood pressure, mmHg	**1st Visit**	72.63 ± 9.58	71.44 ± 10.28	0.0524
	8th Visit	72.73 ± 9.36	73.18 ± 9.48	0.4781
Total cholesterol, mg/dL		196.03 ± 35.51	197.92 ± 34.94	0.4017
Triglyceride, mg/dL		141.82 ± 100.87	141.22 ± 110.73	0.9272
		N (%)	N (%)	
Sex	**Men**	2869 (47.61)	110 (43.65)	0.2175
Smoking status	**Former**	178 (2.95)	5 (1.98)	0.3700
	Current	1387 (23.02)	60 (23.81)	0.7698
Alcohol drinking	**Yes**	3253 (53.98)	131 (51.98)	0.5329
Exercise	**Yes**	5784 (95.98)	242 (96.03)	0.9699
Antidiabetic medication		2042 (33.89)	225 (89.29)	<.0001
Antihypertensive medication		3848 (63.86)	238 (94.44)	<.0001
Type 2 diabetes incident		2968 (49.25)	252 (100.00)	<.0001
Hypertension incident		4082 (67.74)	241 (95.63)	<.0001

Abbreviations: SD, standard deviation; N, number; GRS, genetic risk score; Q quatiles

*p-value for differences between FBS trajectory groups based on T-test or chi-square test

For two SBP patterns (**Figure 17**, no differences between SBP trajectories for age, gender, BMI, FBS, SBP, DBP, total cholesterol and TG at baseline (**Table 13**). And also, differences between FBS trajectories for behavior factors on life-style (smoking status, alcohol drinking, regular exercise) were not shown (**Table 13**).

However, for the participants who took antidiabetic medications, there was a difference between the controlled and uncontrolled groups (33.1% for the controlled groups, 54.9% for the uncontrolled group; p<.0001). Regarding the SBP levels during the follow-up, a greater percentage of uncontrolled participants took antihypertensive medications (60.8% for the controlled group, 91.8% for the uncontrolled group; p<.0001). Furthermore, in the uncontrolled group, the incidence of T2D (47.8% for the controlled group, 73.1% for the uncontrolled group; p<0.0001) and hypertension (64.3% for the controlled group, 97.3% for the uncontrolled group; p<.0001) were higher during the follow-up period (**Table 13**).

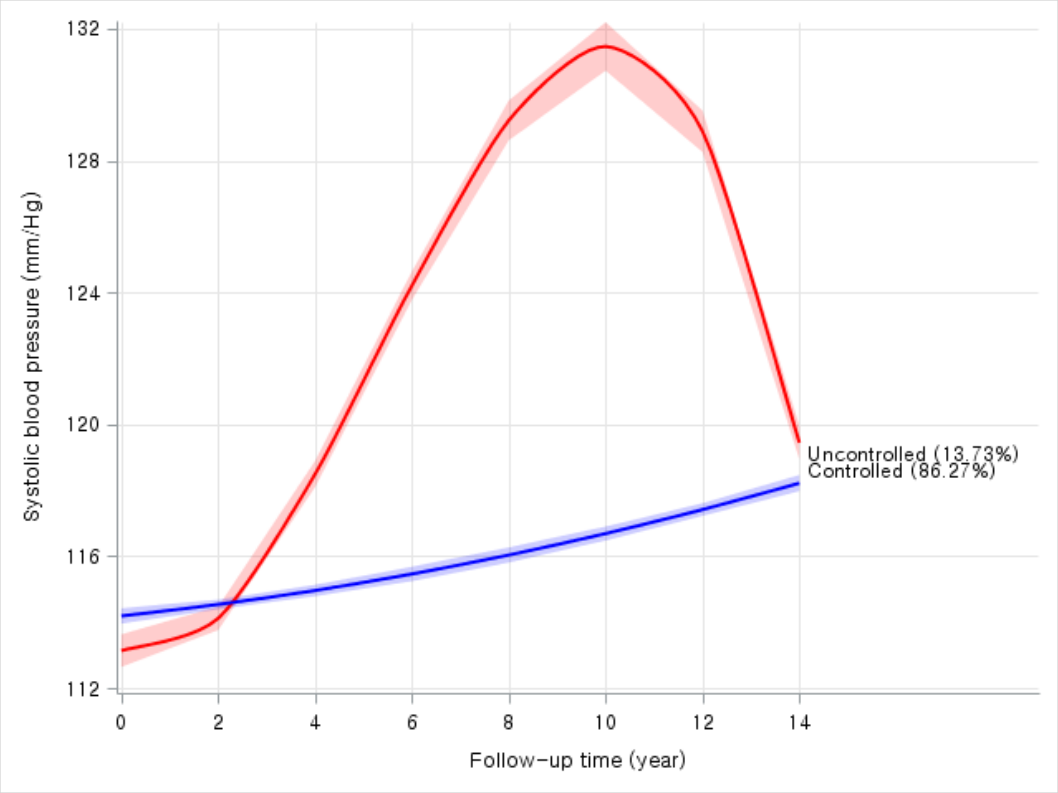


Figure 17. Trajectories of systolic blood pressure in healthy general population

Table 13. General characteristics of the SBP trajectory groups

		Controlled (N=5,416)	Uncontrolled (N=862)	
Subject		Mean ± SD	Mean ± SD	p-value*
Age, year		50.74 ± 8.59	50.38 ± 8.51	0.2562
Body mass index, kg/m²	**1st Visit**	24.20 ± 3.01	24.15 ± 3.02	0.6799
	8th Visit	24.53 ± 3.21	24.49 ± 3.33	0.7629
Fasting blood sugar, mg/dL	**1st Visit**	87.55 ± 8.66	87.35 ± 8.84	0.5275
	8th Visit	101.84 ± 25.28	103.51 ± 31.23	0.1162
Systolic blood pressure, mmHg	**1st Visit**	112.57 ± 14.27	111.98 ± 14.41	0.2620
	8th Visit	119.31 ± 15.12	120.37 ± 15.23	0.0278
Diastolic blood pressure, mmHg	**1st Visit**	72.61 ± 9.62	72.41 ± 9.52	0.5682
	8th Visit	72.65 ± 9.33	73.20 ± 9.50	0.1471
Total cholesterol, mg/dL		196.03 ± 35.17	196.59 ± 37.45	0.6657
Triglyceride, mg/dL		142.40 ± 102.32	137.97 ± 95.45	0.2057
		N (%)	N (%)	
Sex	**Men**	2579 (47.62)	400 (46.40)	0.5072
Smoking status	**Former**	158 (2.92)	25 (2.90)	0.9780
	Current	1231 (22.73)	216 (25.06)	0.1315
Alcohol drinking	**Yes**	2919 (53.90)	465 (53.94)	0.9789
Exercise	**Yes**	5200 (96.01)	826 (95.82)	0.4321
Antidiabetic medication		1794 (33.12)	473 (54.87)	<.0001
Antihypertensive medication		3295 (60.84)	791 (91.76)	<.0001
Type 2 diabetes incident		2590 (47.82)	630 (73.09)	<.0001
Hypertension incident		3484 (64.33)	839 (97.33)	<.0001

Abbreviations: SD, standard deviation; N, number; GRS, genetic risk score; Q quatiles

*p-value for differences between FBS trajectory groups based on T-test or chi-square test

3-3. Associations between the FBS trajectories and the incidence of hypertension

Based on the trajectory analyses, three distinct genetically determined FBS patterns were identified among the patients with T2D during the follow-up period. The risk of developing hypertension in each of the pattern was assessed. In the Cox proportional hazard model, adjusted for age, sex, BMI, smoking behavior, alcohol consumption, and exercise, the uncontrolled group showed a "*reverse U-shape*" pattern and a 1.87-fold risk of developing hypertension [95% confidence interval (CI): 1.64-2.13] compared to controlled group (**Table 14**).

When antihypertensive medications were added to the FBS patterns as covariates in the Cox model, the magnitude was borderline statistically significant for the risk of developing hypertension among those in the uncontrolled group (HR: 1.12, 95% CI: 0.98-1.28 for) (**Table 14**).

In stratified model by sex, similar situations were identified to uncontrolled group adjusted for all-covariates. There was no interaction between men and women (**Table 15**).

Table 14. Association between trajectories of predicted fasting glucose levels and hypertension incidents based on cox proportional-hazards model

	No. of Persons	No. of HTN	Person years, follow-up	HTN incidence Rate per 1000P (95%CI)	HTN		
					Model 1[a] HR (95% CI)	Model 2[b] HR (95% CI)	Model 2[c] HR (95% CI)
Controlled	6026	4082	52940.35	77.11 (74.76-79.51)	1.0	1.0	1.0
Uncontrolled	252	241	1692.65	142.38 (124.97-161.54)	**1.87 (1.64-2.13)**	**1.87 (1.64-2.13)**	**1.12 (0.98-1.28)**
-2 LOG L					71173.201	71193.789	70287.179

Abbreviations: HR, hazard ratio; HTN, hypertension

[a] adjusted for age and sex

[b] adjusted for age, sex, BMI, smoking behavior, alcohol drink and exercise

[c] adjusted for age, sex, BMI, smoking behavior, alcohol drink, exercise and antihypertensives medications

Table 15. Association between trajectories of predicted fasting glucose levels and hypertension incidents by sex based on cox proportional-hazards model

	Men			Women		
	Model 1[a]	Model 2[b]	Model 3[c]	Model 1[a]	Model 2[b]	Model 3[c]
	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)
Controlled	1.0	1.0	1.0	1.0	1.0	1.0
Uncontrolled	1.75 (1.44-2.14)	1.76 (1.44-2.14)	1.06 (0.86-1.30)	1.98 (1.66-2.35)	1.96 (1.65-2.33)	1.18 (0.99-1.41)
P for gender multiplicative interaction			0.4087			
P for gender additive interaction, RERI (95% CI)			0.04 (-0.01-0.09)			
-2 LOG L	30509.457	30501.229	30108.221	34701.242	34691.133	34213.586

Abbreviations: HR, hazard ratio; RERI, relative excess risk due to interaction; CI, confidence interval

[a] adjusted for age

[b] adjusted for age, BMI, smoking behavior, alcohol drink and exercise

[c] adjusted for age, BMI, smoking behavior, alcohol drink, exercise, antihypertensives medications and PRS quartiles

3-2. Associations between SBP trajectories and the incidence of T2D

Based on the trajectory analyses, two clearly distinguished genetically determined SBP trajectories were found in the healthy general population. For each trajectory, the risk of developing T2D was evaluated. In the Cox proportional hazard model, adjusted for age, sex, BMI, smoking behavior, alcohol consumption, and exercise, the uncontrolled group showed a "*reverse U-shape*" pattern and had a significantly higher risk of developing hypertension (HR: 1.88, 95% CI: 1.73-2.06) compared to the controlled group (**Table 16**). After, adding antihypertensive medications to the FBS patterns as covariates in the Cox model, the effect size was demonstrated a slightly significant risk of developing hypertension in the uncontrolled group (HR: 1.27, 95% CI: 1.16-1.38) (**Table 16**).

In stratified model by sex, similar situations were identified to uncontrolled group adjusted for all-covariates. Significant risk of developing T2D was found for men and women of uncontrolled group under the cox model adjusted for all-covariates (HR: 1.28, 95% CI: 1.12-1.45 for men; HR: 1.26, 95% CI: 1.11-1.42 for women). And also, there was no significant multiplicative interaction, but additive interaction was found between men and women (**Table 17**).

Table 16. Association between trajectories of predicted systolic blood pressure and Type 2 diabetes incidents based on cox proportional-hazards model

	No. of Persons	No. of T2D	Person years, follow-up	T2D incidence Rate per 1000P (95%CI)	T2D		
					Model 1[a] HR (95% CI)	Model 2[b] HR (95% CI)	Model 2[c] HR (95% CI)
Controlled	5416	3484	49104.72	70.95 (68.61-73.35)	1.0	1.0	1.0
Uncontrolled	862	839	5528.28	151.77 (141.67-162.39)	**1.88 (1.73-2.06)**	**1.88 (1.73-2.06)**	**1.27 (1.16-1.38)**
-2 LOG L					53560.037	53549.926	52230.228

Abbreviations: HR, hazard ratio; T2D, type 2 diabetes

[a] adjusted for age and sex

[b] adjusted for age, sex, BMI, smoking behavior, alcohol drink, exercise and antidiabetic medications

[c] adjusted for age, sex, BMI, smoking behavior, alcohol drink, exercise, antidiabetic medications and polygenic risk score quartiles

Table 17. Association between trajectories of predicted systolic blood pressure and type 2 diabetes incidents by sex based on cox proportional-hazards model

	Men			Women		
	Model 1[a]	Model 2[b]	Model 3[c]	Model 1[a]	Model 2[b]	Model 3[c]
	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)
Controlled	1.0	1.0	1.0	1.0	1.0	1.0
Uncontrolled	1.92 (1.69-2.18)	1.92 (1.69-2.18)	1.28 (1.12-1.45	1.85 (1.64-2.08)	1.84 (1.63-2.08)	1.26 (1.11-1.42)
P for gender multiplicative interaction	0.7213					
P for gender additive interaction, RERI (95% CI)	0.56 (0.43-0.80)					
-2 LOG L	22971.264	22964.001	22321.196	26137.672	26128.362	25455.191

Abbreviations: HR, hazard ratio; RERI, relative excess risk due to interaction; CI, confidence interval

[a] adjusted for age

[b] adjusted for age, BMI, smoking behavior, alcohol drink, exercise and antidiabetic medications

[c] adjusted for age, BMI, smoking behavior, alcohol drink, exercise, antidiabetic medications and polygenic risk score quartiles

Table 18. Summary for all results from this present study

	MR analysis			Observational analysis		Trajectory analysis
	Significance to 2SLS regression model	**Significance for endogenous test†**	**Significance to pleiotropy under MR-egger**	**Significance to linear regression model**	**Significance to cox proportional hazard model**	**Significance to cox proportional hazard model (Uncontrolled vs. controlled**
FBS→SBP	O	X	X	O	-	-
FBS→HTN	△	X	-	O	X§	X§
SBP→FBS	O	O	O*	O	-	-
SBP→T2D	O	O	-	O	X§	O§

Abbreviations: FBS, fasting blood sugar; SBP, systolic blood pressure; 2SLS, 2-stage least squares; T2D, type 2 diabetes; HTN, hypertension

*Pleiotropy was disappeared after omitted rs671 (top outlier)

†Tests of endogeneity is Wu-Hausman test, Ho: variables are exogenous

§ Significance for association with incidence of T2D / hypertension

IV. DISCUSSION

1. Summary of main findings

In public health, it is very important to account for disease progression by evaluating the interaction of genetic factors with various confounders The current study paid particular attention to the application of MR and trajectory analyses to life course data. In this present study, non-pleiotropy bidirectional causality was identified between fasting blood sugar and systolic blood pressure, and materialized this causality using by trajectory analysis based on life-course approach. This study validated bidirectional causal association results of previous bidirectional MR study in Western, and additionally assessed time-varying changes of causally genetically determined FBS or SBP linked to incident of T2D or hypertension in Korean general population.

The main findings of the 2-stage least squares (2SLS) regression model used in this study highlighted that 1) a 10-mg/dL or 10-mm/Hg genetic increase in FBS or SBP was associated with a 1.63-mmHg increase in SBP ($p=0.005$) or a 11.39-mg/dL increase in FBS ($p<.0001$). The wGRS used as an instrumented variant in the 2SLS regression model included 91 SNPs for FBS, and was deemed not significant according to the endogenous test ($p=0.2596$). In contrast, the wGRS for SBP included 68 SNPs and was assessed to be suitable as an instrumented variant in the 2SLS regression model ($p<0.0001$). Although horizontal pleiotropy existed in the SBP to FBS direction, no pleiotropic result was found from the MR-Egger regression model without outliers. In the sensitivity MR analysis, the "IVW" method, both of the "median" methods (simple and weighted), and the "MR-egger" method, demonstrated a significant bidirectional relationship between FBS and SBP. Rs671 was strong outlier to effected pleiotropic on the direction for SBP to FBS. This bidirectional causality was applied to the FBS or SBP time-varying trajectory analyses to derived particular bidirectional associations according to distinct patterns confirmed by trajectory analyses.

2. Comparison with previous studies

High BP is reported in more than two-thirds of patients with T2D, mainly coexisting with hyperglycemia. Many pathophysiological mechanisms have been identified as the basis for this association [1]. BP is a classical complex genetic trait, with a heritability estimate of 30%–50%. Moreover, hypertension is one of the major cardiovascular risk factors, responsible for up to 50% of cardiovascular morbidity [39]. The importance of BP regulation and the many environmental factors affecting hypertension are well known. However, the origin of essential hypertension is still unclear. Moreover, the heritability of T2D has been estimated to be 40%-70%. The component disorders underlying T2D also have substantial heritability [40-42]. In a Framingham cohort, high BP was associated with a 72% increase in the risk for all-cause death and a 57% increase in the risk for any cardiovascular disease events, making hypertension the most powerful driver of poor cardiovascular outcomes in individuals with T2D [43]. Furthermore, according to population-based longitudinal data, the SBP measurement changed during each visit, and T2D at baseline was a significant predictor of incident hypertension, independent of sex, age, body mass index, and familial diabetes mellitus [10].

A recent study that reported the relationship between FBS and hypertension in a large-scale Korean study showed similar results [44]. This study validated the bidirectional relationship reported in a previous study and established more extensive causal associations through a life course approach.

In the past, T2D has been noted as a major risk factor for cardiovascular disease, and studies have been actively conducted on the relevance or prevention of subsequent hypertension in patients with T2D [45-47]. Since then, other studies have been conducted to identify risk factors at the gene level [39, 48-52]. More recently, there have been attempts to explore the causal inference for highly hereditary T2D and hypertension, not limited to observational studies. In a recent MR study using European meta data, an increase of 1 mmHg in SBP due to genetic risk score including 28 genetic instrument variants was associated with a 2% elevated risk of T2D (OR=1.02, 95% CI, 1.01–1.03, $p=9.1\times10^{-5}$), and there was no pleiotropic expression [12]. Furthermore, using data from the UK Biobank, a bidirectional MR analysis of 134 T2D-related and 233 hypertension-related SNPs in 318,664 individuals of European descent, aged 37 to 73 years , showed that genetically determined T2D was causally-associated with hypertension risk (OR=1.07, 95% CI, 1.04–1.10, $p=3.4\times10^{-7}$), whereas genetically instrumented hypertension was not casually associated with c T2D (OR=0.96, 0.88–1.04, p=0.34)

[13]. However, in this current study, a bidirectional association without pleiotropic bias between genetically elevated FBS and an increased SBP was found. In particular, unlike the results of previous studies that confirmed only the causal relationship in the direction of T2D to hypertension, in this study, the causal association in the direction of SBP to FBS was significant according to the IVW method, the median-based methods (simple and weighted) and MR-Egger method. In the results under MR-Egger method, intercepts were not significant for either directions, namely, FBS to SBP or SBP to FBS; significance for MR-Egger intercept was disappeared in additional analysis after omitting outliers including rs671 for the direction of SBP to FBS. As a member of the alcohol dehydrogenase family known related to alcohol intake and BP in the observational studies [20], future study additionally should assess the role for rs671 on the bidirectional association between BP and FBS.

In recent, some researchers conducted conventional MR analyses based on 2SLS regression model to diverse causal relationships and compared with sensitivity MR analyses like to IVW method, median-based methods (simple and weighted) and MR-egger method [19, 53]. Although there are differences between models in the methodology, it is very meaningful just to compare the results themselves in each model. For this reason, studies are actively underway to compare in-depth conventional MR analysis results, sensitivity MR analysis results, and robust MR analysis results for large-scale biobanks [54, 55].

Meanwhile, in this present study, bidirectional causality was also assessed based on 2SLS regression model using wGRS: an instrumented variant made by 91 SNPs for FBS and 68 SNPs for SBP. There were shown meaningful bidirectional causal relationships between FBS and SBP (including the relationship between T2D and hypertension) assessed in this study using by 2SLS regression model.

Although directions FBS to SBP and FBS to hypertension were significant under 2SLS regression model, but there were non-significant p-value for endogenous test. There were significant results for direction SBP to FBS and SBP to T2D, and results from weak instrument test and endogenous test were significant also. Especially, the relationships for direction SBP to FBS and SBP to T2D in men were more significantly strong than in women. These results not shown when the risk of hypertension or T2D was analyzed according to wGRS quartile group made of genetic instrument variables used in MR analysis among general healthy population. Although this result cannot be vaguely interpreted, it able to be inferred that IVs of the three assumptions of MR analysis are consistent with the assumption that they should not be directly related to outcome, and there was no direct association between genetic instrument variants and future T2D or hypertension. Moreover, in the life-course approach-based trajectory analysis, SBP's uncontrolled pattern showed a significantly 27% higher risk of developing diabetes than the controlled, it able to be thought that was similiar with the results from MR analysis.

Meanwhile, it has been revealed that genetic variables, which have been found to be related to metabolic syndrome and inflammation, are also intertwined in complex pleiotropic genetic relationships [56]. Also, T2D and hypertension shared similar and closely interlinked risk factors, such as endothelial dysfunction, vascular inflammation, arterial remodeling, atherosclerosis, dyslipidemia, and obesity. These risk factors also substantially overlap in cardiovascular complications [57]. Hence, it is very difficult to understand the relationship between T2D and hypertension. To gain a comprehensive understanding, it is necessary to investigate and interpret complex genes and complex mechanisms simultaneously.

There was also a recent evaluation of the risk of death, myocardial infarction, and stroke by forming five risk factors with 271,174 Swedish Type 2 diabetes patients and 1,355,870 controls [58]. The finding was showed that lifestyle and environmental factors are also critical for the development of T2D or hypertension. It has been observed that high genetic susceptibility to hypertension has a high risk of developing cardiovascular disease, but unhealthy lifestyles are also associated with a greater risk of subsequent CVD incidents. These results suggest that genetically predetermined BP and its complications may be, at least to some extent, offset by a healthy lifestyle. These results were also found in this study, when the risk of hypertension and diabetes was analyzed according to genetically determined

FBS and SBP time-varying trajectories in general healthy population. In particular, there was a difference in the risk of subsequent diabetes according to the level of SBP management during the follow-up period. Through this, even with genetically determined SBP, it can be seen that a healthy lifestyle and management are more important than anything else in preventing subsequent T2D. The results of this current further support population-wide efforts to lower the risk of subsequent CVD through lifestyle modifications.

And also, this previous study showed that a hemoglobin A1c (HbA1c) level outside the target range in patients with T2D was the strongest predictor for stroke and acute myocardial infarction. Moreover, smoking was the strongest predictor of all-cause mortality. [58]. These findings warrant further functional investigations. In this present study, changes of predicted HbA1c levels according to follow-up time were additionally assessed using by trajectory analysis, and estimated risk of developed hypertension. Therefore, in the future, it is necessary to extract glycated hemoglobin-related genetic variants to determine the causal relationship under MR analysis, distinguish glycated hemoglobin trajectories over time in patients with T2D, and evaluate the risk of cardiovascular disease.

The results trajectory analysis from this present study, confirming the time-varying patterns of genetically determined FBS and SBP according to the lifestyle specific taking medication for T2D and hypertension in healthy general population, and found that there was a difference in the risk of subsequent T2D or hypertension. Even if the FBS or SBP of middle-aged was uncontrolled and extremely fluctuated, steady management through medication and healthy lifestyle can restore normal levels. However, there was slight different related to future hypertension between poorly controlled FBS group and well-controlled FBS group under the Cox proportional hazard model adjusted for all-covariates (including age, sex, smoking behavior, alcohol drinking, regular exercise and antidiabetic medication). On the other side, the group with poorly controlled SBP patterns during the follow-up period had a 20% higher risk of T2D development independently to the genetic risk factor than the well-controlled group.

Regarding to the 2933 Co-exist cases, accounting for 67.8% of 4,323 hypertensive patients and 91.1% of 3,220 diabetic patients. In fact, considering to person-time, among the 2933 Coexist cases, about 28% of patients with T2D before hypertension occurred, and about 43% of patients with hypertension before T2D. In this respect, among hypertensive patients, the pre-T2D case was 28%, less than the pre-hypertension case (43%) among diabetic patients. However, in the cox model, the magnitude for the relationship was much smaller when antidiabetic medication was further controlled, so it can infer the significant preventive effect of antidiabetic drugs to new-onset hypertension. This result is concordant to previous studies verified antihypertensives effects to antidiabetic Drugs: newer antidiabetes drugs (e.i. sodium glucose cotransporter-2 inhibitors (SGLT2i), glucagon-like peptide-1 receptor agonists (GLP1-RA), and dipeptidyl peptidase-4 inhibitors (DPP4i)) not only have fasting glucose reduction effects, but also BP lowering properties [59, 60]. Research on the effect of blood pressure lowering in relation to the all-cause mortality and cardiovascular disease in diabetic patients has been conducted for a long time [61]. Recently, an individual data meta-analysis, confirm of the result that SBP lowering by 5 mm Hg reduced the risk of T2D by 11% and verification of the effect of each pharmaco-therapeutic type has also conducted: angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers 16%reduced the risk of new-onset T2D in comparison to placebo [62].

The results from previous related studies and this present study suggests that medication management is more important than genetic factors in the relationship between T2D and hypertension according to life-course in the general population, and predisposition for each disease is an important factor in the occurrence of subsequent diseases.

Meanwhile, there is already a study in the Health Professionals Follow-up Study and Nurses' Health Study that investigated the link between genetic predisposition to hypertension and CVD risk in T2D patients, with 29 blood pressure-associated variables showing a consistent link to CVD risk in men and women [63]. In addition, a recent study evaluated sulfonylurea treatment in subjects who failed to achieve blood sugar control with metformin-only therapy, and showed a significant reductions in carriers of G-allele for CDKAL1 rs7756992 related to fasting blood sugar, supporting that treatment response may vary depending on genotype [64]. As a result, in order to understand the relationship between T2D and hypertension, which mainly coexist and are all major risk factors for CVD, more detailed and in-depth research should be conducted considering predisposition and drug type in the future.

3. Plausible mechanisms

The results of this study showed that an increase in FBS based on heredity affects SBP and vice versa. These findings may partly explain the link between T2D and new onset hypertension or between hypertension and new onset T2D.

It is unclear whether the pathological association between BP and diabetes is causal. Results from a previous network meta-analysis of randomized trials showed that renin-angiotensin system (RAS) activation is a causal risk of new onset diabetes, but not BP [65]. Moreover, previous analyses suggested that a causal relationship may exist between chronic inflammatory mediators, especially interleukin-6 and incident T2D [66, 67]. Chronic inflammation is characterized by obesity [68] and elevated BP [69], risk factors for diabetes, and is reduced by the renin-angiotensin-system (RAS) inhibition (e.g., initial medications for the management of hypertension) [70]. Thus, chronic inflammation may, in part, mediate the relationship between risk factors (obesity and hypertension) and the onset of diabetes. Alternatively, endothelial dysfunction may link elevated BP and diabetes [50]. T2D and hypertension have similar and closely interlinked risk factors including endothelial dysfunction [50], vascular inflammation, arterial remodeling, atherosclerosis, dyslipidemia, and obesity. These risk factors also substantially overlap in cardiovascular complications [57]. Recently, genetic

variables related to metabolic syndrome and inflammation have also been found to contribute to the complex pleiotropic genetic relationships [56]. Hence, it is very difficult to understand the relationship between T2D and hypertension. To gain an in-depth understanding, it is necessary to study and interpret complex genes and complex mechanisms simultaneously. This current study was also based on the pathological assumptions reported in previous studies. Assuming causality, it may be suggested that individual and population-based efforts to lower BP may also lower the incidence of diabetes. Considering this, the present study contributed further evidence.

4. Strengths and limitations

4-1. Strengths

The main purpose of this study was to improve the MR analysis method of previous studies targeting Western populations, derive results for Koreans, and provide more detailed evidence of the causal relationship between hypertension and T2D. This study has the main strength of being the first study attempted with this purpose.

It is the first study to validate a previous MR study and confirm the bidirectional causal association between FBS and SBP in the Korean population. Furthermore, this study is very meaningful in terms of methodology for finding causality by comparing the results of different methods themselves in MR analysis, and it is the first study to make such an attempt on Koreans.

In addition, the first attempt to find the causal relationship between hypertension and T2D using the large number of IVs based on MR analysis in the Korean general population.

Lastly, the main evaluations were expanded through a life course approach using trajectory analyses as well as the MR analysis. Regarding these point, the additional strength of this study is that it is the first study to bring causality at the genetic level to a life course concept.

4-2. Limitations

Despite these strengths, there were several limitations of this current study. First, there were few participants in the MR analysis. SNPs extracted from the large-scale biobank (i.e. KCPS-II) were used as candidate instrument genetic variables to compensate for this limitation. The number of patterns and the number of stratified

subjects according to each pattern for FBS and SBP classified after trajectory analyses were also not sufficient. Moreover, all trajectory analyses were performed under the basic assumptions for trajectory analysis, and all participants were properly classified according to optimal models. For the FBS trajectories, even when the number of groups was set to 2, the proportion of the group with the smallest number of individuals was below 3 (which violates the assumption), so the p-value for grouping, and the BIC value for the model, were considered as a whole, and then, finally classified into two groups (controlled and uncontrolled). Although a bidirectional causal relationship between FBS and SBP levels was confirmed, it is somewhat leaps and bounds to link them with the results of trajectory analysis. Furthermore, it is difficult to interpret the results using the life course approach because diverse metabolic factors may be complexly associated between T2D and hypertension. Therefore, if Trajectory-based results are considered completely different stories, a new research model capable of causal inference for new-onset outcome needs to be presented to link causal results from gene-based MR studies to life-source-based results, and future studies including various and complex metabolic factors are needed. Finally, since this study is limited to middle-aged Koreans, it is difficult to g eneralize the research results.

V. CONCLUSION

In summary, a bidirectional causal association between FBS and SBP was identified based on the life course approach. In particular, genetically determined SBP levels significantly affected FBS increases, and the results were strong in men. Different distinct time-varying patterns of FBS and SBP based on life-course approach were attempted using by trajectory analysis, and the risk of future diabetes was significantly higher if the SBP level was not well controlled by drugs. In the future, elaborative large biobank studies including countless genetic variants and different environmental interactions are needed to validate the bidirectional causal association between FBS and SBP using the life course approach.

.

REFERENCES

1. Ferrannini, E. and W.C. Cushman, *Diabetes and hypertension: the bad companions.* Lancet, 2012. **380**(9841): p. 601-10.
2. Roth, G.A., et al., *Global and regional patterns in cardiovascular mortality from 1990 to 2013.* Circulation, 2015. **132**(17): p. 1667-78.
3. Roth, G.A., et al., *Global, Regional, and National Burden of Cardiovascular Diseases for 10 Causes, 1990 to 2015.* J Am Coll Cardiol, 2017. **70**(1): p. 1-25.
4. KCDC, *Korea health statistics 2020 Korea national health and nutrition examination survey*. 2020.
5. Goff, D.C., Jr., et al., *Insulin sensitivity and the risk of incident hypertension: insights from the Insulin Resistance Atherosclerosis Study.* Diabetes Care, 2003. **26**(3): p. 805-9.
6. Lai, T.S., G.C. Curhan, and J.P. Forman, *Insulin resistance and risk of incident hypertension among men.* J Clin Hypertens (Greenwich), 2009. **11**(9): p. 483-90.
7. Levin, G., et al., *Glucose, insulin, and incident hypertension in the multi-ethnic study of atherosclerosis.* Am J Epidemiol, 2010. **172**(10): p. 1144-54.
8. Ghaderian, S.B. and S.S. Beladi-Mousavi, *The role of diabetes mellitus and hypertension in chronic kidney disease.* J Renal Inj Prev, 2014. **3**(4): p. 109-10.
9. Verdecchia, P., et al., *Adverse prognostic significance of new diabetes in treated hypertensive subjects.* Hypertension, 2004. **43**(5): p. 963-9.
10. Tsimihodimos, V., et al., *Hypertension and Diabetes Mellitus: Coprediction and Time Trajectories.* Hypertension, 2018. **71**(3): p. 422-428.
11. Colussi, G., A. Da Porto, and A. Cavarape, *Hypertension and type 2 diabetes: lights and shadows about causality.* J Hum Hypertens, 2020. **34**(2): p. 91-93.

12. Aikens, R.C., et al., *Systolic Blood Pressure and Risk of Type 2 Diabetes: A Mendelian Randomization Study.* Diabetes, 2017. **66**(2): p. 543-550.
13. Sun, D., et al., *Type 2 Diabetes and Hypertension.* Circ Res, 2019. **124**(6): p. 930-937.
14. Kim, Y. and B.G. Han, *Cohort Profile: The Korean Genome and Epidemiology Study (KoGES) Consortium.* Int J Epidemiol, 2017. **46**(2): p. e20.
15. Jee, Y.H., et al., *Cohort Profile: The Korean Cancer Prevention Study-II (KCPS-II) Biobank.* Int J Epidemiol, 2018. **47**(2): p. 385-386f.
16. Han, N., J.M. Oh, and I.W. Kim, *Combination of Genome-Wide Polymorphisms and Copy Number Variations of Pharmacogenes in Koreans.* J Pers Med, 2021. **11**(1).
17. Heid, I.M., et al., *Meta-analysis identifies 13 new loci associated with waist-hip ratio and reveals sexual dimorphism in the genetic basis of fat distribution.* Nat Genet, 2010. **42**(11): p. 949-60.
18. Kong, S.H., et al., *Identification of Novel Genetic Variants Related to Trabecular Bone Score in Community-Dwelling Older Adults.* Endocrinol Metab (Seoul), 2020. **35**(4): p. 801-810.
19. Huang, T., et al., *Conventional and Bidirectional Genetic Evidence on Resting Heart Rate and Cardiometabolic Traits.* J Clin Endocrinol Metab, 2022. **107**(4): p. e1518-e1527.
20. Jee, Y.H., et al., *Alcohol Intake and Serum Glucose Levels from the Perspective of a Mendelian Randomization Design: The KCPS-II Biobank.* PLoS One, 2016. **11**(9): p. e0162930.
21. Spiller, W., et al., *Precision Medicine and Cardiovascular Health: Insights from Mendelian Randomization Analyses.* Korean Circ J, 2020. **50**(2): p. 91-111.
22. Lee, K. and C.Y. Lim, *Mendelian Randomization Analysis in Observational Epidemiology.* J Lipid Atheroscler, 2019. **8**(2): p. 67-77.
23. Smith, G.D. and S. Ebrahim, *'Mendelian randomization': can*

genetic epidemiology contribute to understanding environmental determinants of disease? Int J Epidemiol, 2003. **32**(1): p. 1-22.
24. Smith, G.D. and S. Ebrahim, *Mendelian randomization: prospects, potentials, and limitations.* Int J Epidemiol, 2004. **33**(1): p. 30-42.
25. Bowden, J., G. Davey Smith, and S. Burgess, *Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression.* Int J Epidemiol, 2015. **44**(2): p. 512-25.
26. Bowden, J., et al., *Consistent Estimation in Mendelian Randomization with Some Invalid Instruments Using a Weighted Median Estimator.* Genet Epidemiol, 2016. **40**(4): p. 304-14.
27. Burgess, S., A. Butterworth, and S.G. Thompson, *Mendelian randomization analysis with multiple genetic variants using summarized data.* Genet Epidemiol, 2013. **37**(7): p. 658-65.
28. Burgess, S. and S.G. Thompson, *Interpreting findings from Mendelian randomization using the MR-Egger method.* Eur J Epidemiol, 2017. **32**(5): p. 377-389.
29. Martens, E.P., et al., *Instrumental variables: application and limitations.* Epidemiology, 2006. **17**(3): p. 260-7.
30. Palmer, T.M., et al., *Instrumental variable estimation of causal risk ratios and causal odds ratios in Mendelian randomization analyses.* Am J Epidemiol, 2011. **173**(12): p. 1392-403.
31. Ziegler, A., F. Pahlke, and I.R. König, *Comments on 'Mendelian randomization: using genes as instruments for making causal inferences in epidemiology' by Debbie A. Lawlor, R. M. Harbord, J. A. Sterne, N. Timpson and G. Davey Smith, Statistics in Medicine, DOI: 10.1002/sim.3034.* Stat Med, 2008. **27**(15): p. 2974-6; author reply 2976-8.
32. Young Ho Lee, *Overview of Mendelian Randomization Analysis.* Journal of Rheumatic Diseases, 2020. **27**(4): p. 241-246.
33. Buscot, M.J., et al., *Distinct child-to-adult body mass index trajectories are associated with different levels of adult*

cardiometabolic risk. Eur Heart J, 2018. **39**(24): p. 2263-2270.
34. Jeon, J., K.J. Jung, and S.H. Jee, *Waist circumference trajectories and risk of type 2 diabetes mellitus in Korean population: the Korean genome and epidemiology study (KoGES).* BMC Public Health, 2019. **19**(1): p. 741.
35. Jeon, J., et al., *Changes in secondhand smoke exposure levels and risk of type 2 diabetes in middle age: the Korean Genome and Epidemiology Study (KoGES).* BMJ Open Diabetes Res Care, 2019. **7**(1): p. e000859.
36. Zheng, H., D. Tumin, and Z. Qian, *Obesity and mortality risk: new findings from body mass index trajectories.* Am J Epidemiol, 2013. **178**(11): p. 1591-9.
37. Correia, K. and P.L. Williams, *Estimating the Relative Excess Risk Due to Interaction in Clustered-Data Settings.* Am J Epidemiol, 2018. **187**(11): p. 2470-2480.
38. Lee, H.A., H. Park, and Y.S. Hong, *Sex Differences in the Effects of CDKAL1 Variants on Glycemic Control in Diabetic Patients: Findings from the Korean Genome and Epidemiology Study.* Diabetes Metab J, 2022. **46**(6): p. 879-889.
39. Ehret, G.B. and M.J. Caulfield, *Genes for blood pressure: an opportunity to understand hypertension.* Eur Heart J, 2013. **34**(13): p. 951-61.
40. Almgren, P., et al., *Heritability and familiality of type 2 diabetes and related quantitative traits in the Botnia Study.* Diabetologia, 2011. **54**(11): p. 2811-9.
41. Avery, A.R. and G.E. Duncan, *Heritability of Type 2 Diabetes in the Washington State Twin Registry.* Twin Res Hum Genet, 2019. **22**(2): p. 95-98.
42. Goodarzi, M.O. and J.I. Rotter, *Genetics Insights in the Relationship Between Type 2 Diabetes and Coronary Heart Disease.* Circ Res, 2020. **126**(11): p. 1526-1548.
43. Chen, G., et al., *Cardiovascular outcomes in framingham participants with diabetes: the importance of blood pressure.*

Hypertension, 2011. **57**(5): p. 891-7.
44. Ahn, S.K., et al., *Incidence Hypertension and Fasting Blood Glucose from Real-World Data: Retrospective Cohort for 7-Years Follow-Up.* Int J Environ Res Public Health, 2021. **18**(4).
45. Kannel, W.B. and D.L. McGee, *Diabetes and cardiovascular risk factors: the Framingham study.* Circulation, 1979. **59**(1): p. 8-13.
46. Taylor, K.S., et al., *All-cause and cardiovascular mortality in middle-aged people with type 2 diabetes compared with people without diabetes in a large U.K. primary care database.* Diabetes Care, 2013. **36**(8): p. 2366-71.
47. Rawshani, A., et al., *Risk Factors, Mortality, and Cardiovascular Outcomes in Patients with Type 2 Diabetes.* N Engl J Med, 2018. **379**(7): p. 633-644.
48. Amrani-Midoun, A., et al., *Genetic association study between T-786C NOS3 polymorphism and essential hypertension in an Algerian population of the Oran city.* Diabetes Metab Syndr, 2019. **13**(2): p. 1317-1320.
49. Langenberg, C. and L.A. Lotta, *Genomic insights into the causes of type 2 diabetes.* Lancet, 2018. **391**(10138): p. 2463-2474.
50. Meigs, J.B., et al., *Biomarkers of endothelial dysfunction and risk of type 2 diabetes mellitus.* Jama, 2004. **291**(16): p. 1978-86.
51. Meigs, J.B., *The Genetic Epidemiology of Type 2 Diabetes: Opportunities for Health Translation.* Curr Diab Rep, 2019. **19**(8): p. 62.
52. Wang, Y. and J.G. Wang, *Genome-Wide Association Studies of Hypertension and Several Other Cardiovascular Diseases.* Pulse (Basel), 2019. **6**(3-4): p. 169-186.
53. Lankester, J., et al., *Alcohol use and cardiometabolic risk in the UK Biobank: A Mendelian randomization study.* PLoS One, 2021. **16**(8): p. e0255801.
54. Larsson, S.C., et al., *Alcohol Consumption and Cardiovascular Disease: A Mendelian Randomization Study.* Circ Genom Precis Med, 2020. **13**(3): p. e002814.

55. Linden, A.B., et al., *Genetic associations of adult height with risk of cardioembolic and other subtypes of ischemic stroke: A mendelian randomization study in multiple ancestries.* PLoS Med, 2022. **19**(4): p. e1003967.
56. Kraja, A.T., et al., *Pleiotropic genes for metabolic syndrome and inflammation.* Mol Genet Metab, 2014. **112**(4): p. 317-38.
57. Petrie, J.R., T.J. Guzik, and R.M. Touyz, *Diabetes, Hypertension, and Cardiovascular Disease: Clinical Insights and Vascular Mechanisms.* Can J Cardiol, 2018. **34**(5): p. 575-584.
58. Pazoki, R., et al., *Genetic Predisposition to High Blood Pressure and Lifestyle Factors: Associations With Midlife Blood Pressure Levels and Cardiovascular Events.* Circulation, 2018. **137**(7): p. 653-661.
59. Mamakou, V., et al., *Antidiabetic Drugs as Antihypertensives: New Data on the Horizon.* Curr Vasc Pharmacol, 2017. **16**(1): p. 70-78.
60. Shaikh, A., *A Practical Approach to Hypertension Management in Diabetes.* Diabetes Ther, 2017. **8**(5): p. 981-989.
61. Emdin, C.A., et al., *Blood pressure lowering in type 2 diabetes: a systematic review and meta-analysis.* Jama, 2015. **313**(6): p. 603-15.
62. Nazarzadeh, M., et al., *Blood pressure lowering and risk of new-onset type 2 diabetes: an individual participant data meta-analysis.* Lancet, 2021. **398**(10313): p. 1803-1810.
63. Qi, Q., et al., *Genetic predisposition to high blood pressure associates with cardiovascular complications among patients with type 2 diabetes: two independent studies.* Diabetes, 2012. **61**(11): p. 3026-32.
64. Schroner, Z., et al., *Variation in CDKAL1 gene is associated with therapeutic response to sulphonylureas.* Physiol Res, 2012. **61**(2): p. 177-83.
65. Elliott, W.J. and P.M. Meyer, *Incident diabetes in clinical trials of antihypertensive drugs: a network meta-analysis.* Lancet,

2007. **369**(9557): p. 201-7.
66. Pradhan, A.D., et al., *C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus.* Jama, 2001. **286**(3): p. 327-34.
67. Swerdlow, D.I., et al., *The interleukin-6 receptor as a target for prevention of coronary heart disease: a mendelian randomisation analysis.* Lancet, 2012. **379**(9822): p. 1214-24.
68. Dandona, P., A. Aljada, and A. Bandyopadhyay, *Inflammation: the link between insulin resistance, obesity and diabetes.* Trends Immunol, 2004. **25**(1): p. 4-7.
69. Chamarthi, B., et al., *Inflammation and hypertension: the interplay of interleukin-6, dietary sodium, and the renin-angiotensin system in humans.* Am J Hypertens, 2011. **24**(10): p. 1143-8.
70. Ridker, P.M., et al., *Valsartan, blood pressure reduction, and C-reactive protein: primary report of the Val-MARC trial.* Hypertension, 2006. **48**(1): p. 73-9.

Appendix 1. Bidirectional association between FBS and SBP using weighted genetic risk score (91 SNPs for FBS / 68 SNPs for SBP) as a genetic instrument variants based on 2-stage least squares (2SLS) regression model

			Observational multivariable regression analysis* X-Y			Mendelian randomization analysis				
			β	SE	p	F-statistic G-X	P for endogenous test†	β	SE	p
FBS→ SBP	PRS for FBS (N=91)	**Total***	0.171	0.015	<.0001	157.39	0.2596	0.167	0.063	0.007
		Men	0.162	0.025	<.0001	109.25	0.3213	0.128	0.064	0.046
		Women	0.174	0.018	<.0001	51.65	0.4608	0.233	0.127	0.066
SBP→ FBS	PRS for SBP (N=68)	**Total***	**0.090**	**0.008**	**<.0001**	**40.64**	**<.0001**	**1.137**	**0.252**	**<.0001**
		Men	**0.064**	**0.010**	**<.0001**	**39.54**	**<.0001**	**1.431**	**0.316**	**<.0001**
		Women	0.119	0.012	<.0001	10.16	0.1843	0.636	0.384	0.097

Abbreviations: SE, standard error

*Adjusted for age and sex (Adjusted for age only when model was stratified into men and women)

†Tests of endogeneity is Wu-Hausman test, Ho: variables are exogenous

Appendix 2. Bidirectional causal association on SBP to FBS direction from sensitivity mendelian Randomization analysis using weighted genetic risk score without outliers as a genetic instrument variable based on based on 2-stage least squares (2SLS) regression model

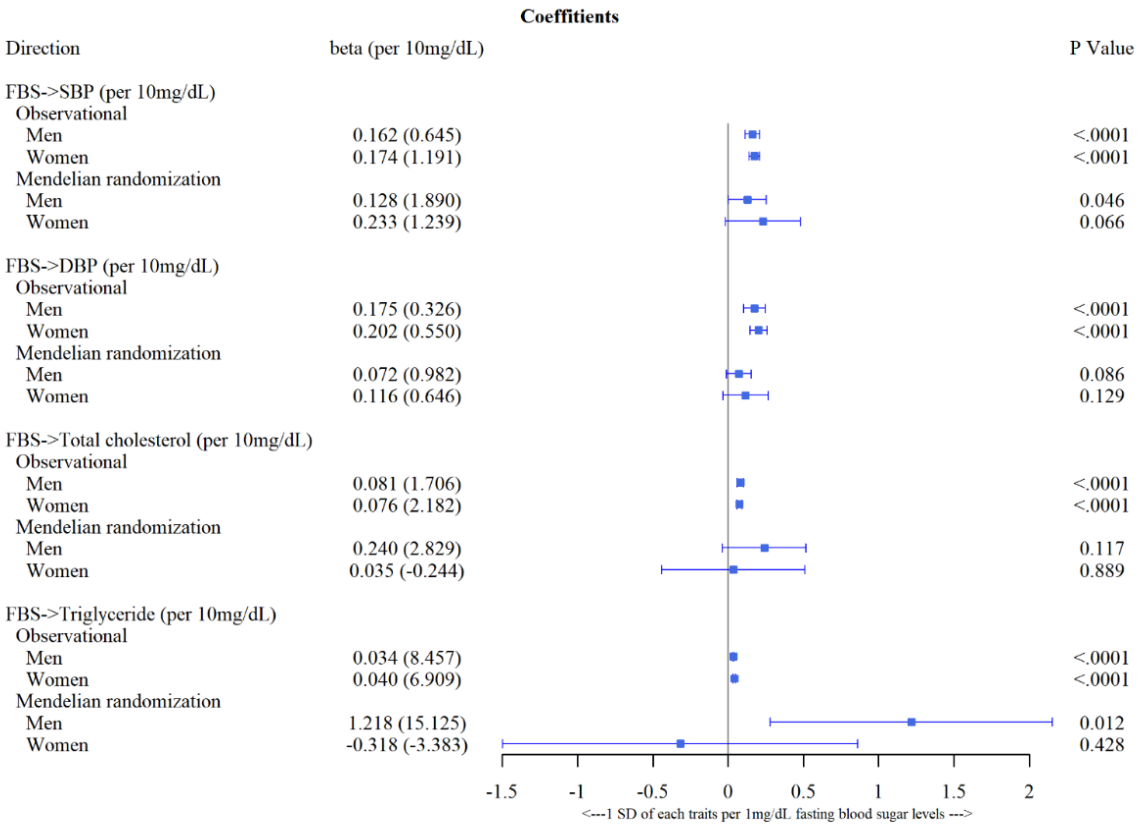
			Observational multivariable regression analysis X-Y			Mendelian randomization analysis				
			β	SE	p	F-statistic G-X	P for endogenous test†	β	SE	p
SBP→FBS (per 10 mm/Hg)	PRS for SBP (N=65)	**Total***	1.706	0.148	<.0001	33.72	<.0001	11.535	2.196	<.0001
		Men	1.625	0.249	<.0001	25.98	<.0001	17.582	3.433	<.0001
		Women	1.741	0.179	<.0001	11.09	0.1035	6.046	2.796	0.031
SBP→DBP (per 10 mm/Hg)	PRS for SBP (N=65)	**Total***	5.304	0.042	<.0001	33.72	0.0153	6.595	1.045	<.0001
		Men	5.477	0.065	<.0001	25.98	0.1185	7.333	1.481	<.0001
		Women	5.140	0.055	<.0001	11.09	0.0719	6.098	1.456	<.0001

Abbreviations: SE, standard error

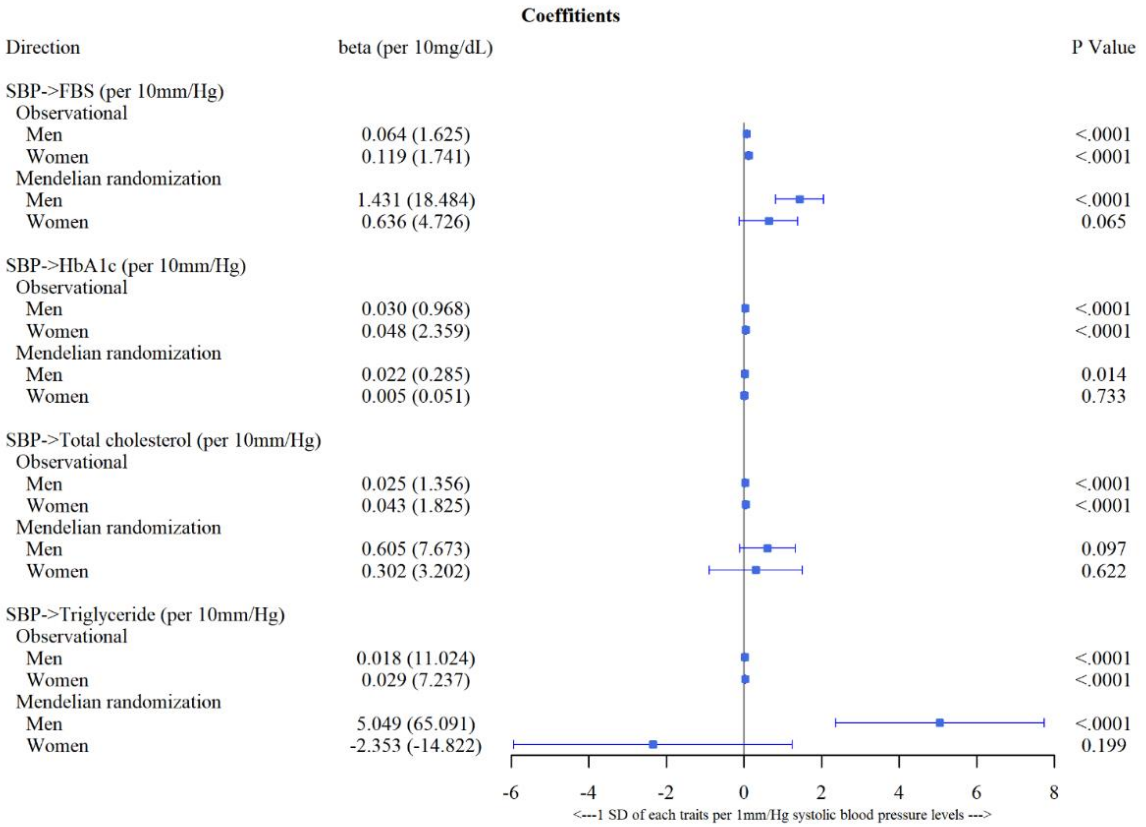
*Adjusted for age and sex (Adjusted for age only when model was stratified into men and women)

†Tests of endogeneity is Wu-Hausman test, Ho: variables are exogenous

Appendix 3. The effects of FBS on metabolic components from Observational and Mendelian randomization analyses testing by sex



Appendix 4. The effects of SBP on metabolic components from Observational and Mendelian randomization analyses testing by sex



Appendix 5. Association between wGRS of FBS and Hypertension incident by sex on cox proportional-hazards model

	Men			Women		
	Model 1[a] HR (95% CI)	Model 2[b] HR (95% CI)	Model 2[c] HR (95% CI)	Model 1[a] HR (95% CI)	Model 2[b] HR (95% CI)	Model 2[c] HR (95% CI)
Q1	**1.0**	**1.0**	**1.0**	**1.0**	**1.0**	**1.0**
Q2	0.98 (0.87-1.10)	0.97 (0.86-1.10)	0.95 (0.84-1.07)	0.96 (0.85-1.08)	0.95 (0.85-1.07)	0.94 (0.84-1.06)
Q3	0.89 (0.79-1.01)	0.88 (0.79-1.00)	0.88 (0.77-0.99)	1.03 (0.92-1.15)	1.03 (0.92-1.16)	1.01 (0.90-1.14)
Q4	0.88 (0.77-0.99)	0.86 (0.76-0.98)	0.91 (0.80-1.03)	1.04 (0.93-1.17)	1.04 (0.92-1.17)	1.00 (0.89-1.13)
P for trend	**0.0135**	**0.0444**	**0.0785**	**0.3005**	**0.5972**	**0.5234**
P for interaction			**0.0731**			
-2 LOG L	30528.977	30515.033	30095.347	34747.769	34737.063	34214.809

Abbreviations: HR, hazard ratio
[a] adjusted for age
[b] adjusted for age, BMI, smoking behavior, alcohol drink and exercise
[c] adjusted for age, BMI, smoking behavior, alcohol drink, exercise and antidiabetic medications

Appendix 6. Association between wGRS of SBP and type 2 diabetes incident by sex based on cox proportional-hazards model-

	Men			Women		
	Model 1[a] HR (95% CI)	Model 2[b] HR (95% CI)	Model 2[c] HR (95% CI)	Model 1[a] HR (95% CI)	Model 2[b] HR (95% CI)	Model 2[c] HR (95% CI)
Q1	**1.0**	**1.0**	**1.0**	**1.0**	**1.0**	**1.0**
Q2	0.94 (0.82-1.09)	0.93 (0.81-1.07)	0.95 (0.82-1.10)	1.10 (0.96-1.26)	1.10 (0.96-1.26)	1.09 (0.95-1.25)
Q3	0.98 (0.86-1.13)	0.97 (0.84-1.12)	0.97 (0.84-1.13)	1.06 (0.93-1.22)	1.08 (0.94-1.23)	1.09 (0.94-1.25)
Q4	0.83 (0.72-0.95)	0.82 (0.71-0.95)	0.85 (0.73-0.99)	1.01 (0.88-1.16)	1.02 (0.89-1.18)	1.02 (0.89-1.18)
P for trend	**0.0225**	**0.0168**	**0.0658**	**0.9150**	**0.8785**	**0.8252**
P for interaction			**0.2641**			
-2 LOG L	23049.777	22314.506	22324.291	26224.420	26213.028	25464.224

Abbreviations: HR, hazard ratio

[a] adjusted for age

[b] adjusted for age, BMI, smoking behavior, alcohol drink and exercise

[c] adjusted for age, BMI, smoking behavior, alcohol drink, exercise and antihypertensive medications

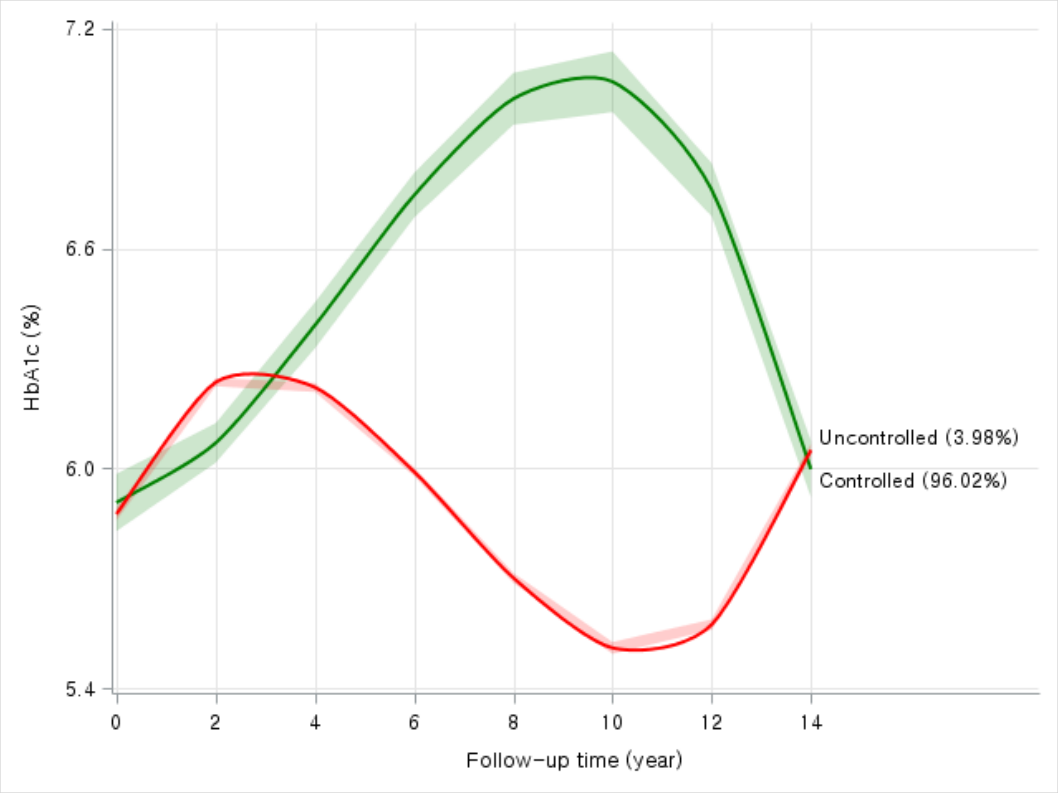
Appendix 7. General characteristics of hemoglobin A1c trajectories

		Controlled (N=6,028)	Uncontrolled (N=250)	
Subject		Mean ± SD	Mean ± SD	p-value
Age, year		51.46 ± 8.31	50.66 ± 8.59	0.1374
Body mass index, kg/m^2	**1st Visit**	24.20 ± 3.23	24.19 ± 3.00	0.9523
	8th Visit	24.27 ± 3.16	25.54 ± 3.24	0.2056
Fasting blood sugar, mg/dL	**1st Visit**	86.89 ± 9.00	87.56 ± 8.67	0.2527
	8th Visit	102.55 ± 29.94	102.14 ± 26.31	0.8205
HbA1c, %	**1st Visit**	**5.52 ± 0.34**	**5.54 ± 0.34**	**0.3849**
	8th Visit	**5.95 ± 1.06**	**5.95 ± 0.93**	**0.9731**
Systolic blood pressure, mmHg	**1st Visit**	112.27 ± 14.00	112.50 ± 14.30	0.8020
	8th Visit	119.69 ± 13.35	119.28 ± 15.26	0.6794
Diastolic blood pressure, mmHg	**1st Visit**	72.33 ± 9.77	72.60 ± 9.60	0.6661
	8th Visit	71.97 ± 8.73	72.81 ± 9.40	0.1495
Total cholesterol, mg/dL		199.04 ± 36.73	195.98 ± 35.44	0.1970
Triglyceride, mg/dL		134.89 ± 88.92	142.08 ± 101.75	0.2716
		N (%)	N (%)	
Sex	**Men**	2870 (47.61)	109 (43.60)	0.2133
Smoking status	**Former**	177 (2.94)	6 (2.40)	0.6214
	Current	1390 (23.06)	57 (22.80)	0.9241
Alcohol drinking	**Yes**	3261 (54.10)	123 (49.20)	0.1280
Exercise	**Yes**	5787 (96.00)	239 (95.60)	0.7510
Antidiabetic medication		2043 (33.89)	224 (89.60)	<.0001
Antihypertensive medication		3851 (63.89)	235 (94.00)	<.0001
Type 2 diabetes incident		2970 (49.27)	250 (100.00)	<.0001
Hypertension incident		4085 (67.77)	238 (95.20)	<.0001

Abbreviations: SD, standard deviation; N, number;

[a] p-value for differences between FBS trajectory groups based on T-test or chi-square test

Appendix 8. Trajectories of hemoglobin A1c



Appendix 9. Association between trajectories of hemoglobin A1c and hypertension based on cox proportional-hazards model

	No. of Persons	No. of HTN	Person years, follow-up	HTN incidence Rate per 1000P (95%CI)	HTN		
					Model 1[a]	Model 2[b]	Model 2[c]
					HR (95% CI)	HR (95% CI)	HR (95% CI)
Controlled	6028	4085	52922.78	77.19 (74.84-79.59)	1.0	1.0	1.0
Uncontrolled	250	238	1710.22	151.77 (141.67-162.39)	**1.81 (1.59-2.07)**	**1.81 (1.60-2.07)**	**1.09 (0.95-1.24)**
-2 LOG L					71180.736	71174.458	70288.407

Abbreviations: HR, hazard ratio; HTN, hypertension

[a] adjusted for age and sex

[b] adjusted for age, sex, BMI, smoking behavior, alcohol drink and exercise

[c] adjusted for age, sex, BMI, smoking behavior, alcohol drink, exercise and antidiabetic medications

Appendix 10. Association between trajectories of hemoglobin A1c and hypertension by sex based on cox proportional-hazards model

	Men			Women		
	Model 1[a]	Model 2[b]	Model 3[c]	Model 1[a]	Model 2[b]	Model 3[c]
	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)
Controlled	1.0	1.0	1.0	1.0	1.0	1.0
Uncontrolled	**1.88 (1.54-2.29)**	**1.89 (1.55-2.30)**	**1.15 (0.94-1.41)**	**1.76 (1.48-2.10)**	**1.76 (1.47-2.10)**	**1.04 (0.87-1.25)**
P for gender multiplicative interaction	0.5189					
P for gender additive interaction, RERI (95% CI)	**-0.07 (-0.17-0.02)**					
-2 LOG L	30502.665	30494.176	30106.736	34716.311	34705.691	34216.459

Abbreviations: HR, hazard ratio; RERI, relative excess risk due to interaction; CI, confidence interval

[a] adjusted for age and sex

[b] adjusted for age, sex, BMI, smoking behavior, alcohol drink and exercise

[c] adjusted for age, sex, BMI, smoking behavior, alcohol drink, exercise and antidiabetic medications

Appendix 11. Summary of 2-stage least squares (2SLS) regression model

"two-stage least squares" would be running OLS two times. Assume we want to estimate the coefficients of the linear model.

$Yi = \beta 0 + \beta 1X1_i + \cdots + \beta pXp_i + \varepsilon_i,$

but some of the variables X_{ji} are correlated with the error term. OLS estimation of this equation will be biased and inconsistent, as we have already seen. When instrument variables (are not themselves covariates) are existed in the linear model, where each satisfies the following conditions:

1. First stage: Z affects X.

2. Independence: Z is uncorrelated with ε.

3. Exclusion restriction: Z only affects Y through its effect on X.

Under these conditions, any exogenous X variable (i.e., any that is uncorrelated with the error term) can be included in Z. One additional instrument per endogenous variable is just need at least. The two-stage least squares estimator of β is the following procedure:

1. Regress each X j on Z and save the predicted values, $\hat{X}_j$.

   If X_j is included in Z, $\hat{X}_j = X_j$ would be given.

2. Estimate β via the OLS estimate of the regression model

   $$Y_i = \beta 0 + \beta 1 \hat{X}1_i + \cdots + \beta p \hat{X}p_i + \varepsilon_i$$

This is obviously easy to implement, and it allows us to incorporate exogenous covariates, multiple endogenous variables, and more instruments than endogenous variables.

Appendix 12. The following is a series of commands that illustrates summary of the range of the MendelianRandomization package aimed at causal or first-time users of the R software environment

```
# lines beginning with "#" are comments and are not run
install.packages("MendelianRandomization")
# installation of the package is only necessary at first use
library(MendelianRandomization)

######### 91snps for FBS, 68snps for SBP: bidirectional MR

setwd("F:/Analysis")

fbs_koges_be<-read.table("F:/Analysis/kcps2_fbsto_beta.csv",sep=",",header=T)
fbs_koges_se<-read.table("F:/Analysis/kcps2_fbsto_se.csv",sep=",",header=T)
sbp_koges_be<-read.table("F:/Analysis/kcps2_tosbp_beta.csv",sep=",",header=T)
sbp_koges_se<-read.table("F:/Analysis/kcps2_tosbp_se.csv",sep=",",header=T)

fbs_be=unlist(fbs_koges_be)
names(fbs_be)=NULL
fbs_se=unlist(fbs_koges_se)
names(fbs_se)=NULL
sbp_be=unlist(sbp_koges_be)
names(sbp_be)=NULL
sbp_se=unlist(sbp_koges_se)
names(sbp_se)=NULL

MRdata_fbs_sbp<- mr_input(bx = fbs_be, bxse = fbs_se, by = sbp_be, byse = sbp_se)
mr_allmethods(MRdata_fbs_sbp, method ="main")
mr_plot(mr_allmethods(MRdata_fbs_sbp, method = "main"))
mr_plot(MRdata_fbs_sbp, orientate = TRUE, line = "egger")


sbp_koges_be<-read.table("F:/Analysis/kcps2_sbpto_beta.csv",sep=",",header=T)
sbp_koges_se<-read.table("F:/Analysis/kcps2_sbpto_se.csv",sep=",",header=T)
fbs_koges_be<-read.table("F:/Analysis/kcps2_tofbs_beta.csv",sep=",",header=T)
fbs_koges_se<-read.table("F:/Analysis/kcps2_tofbs_se.csv",sep=",",header=T)

sbp_be=unlist(sbp_koges_be)
names(sbp_koges_be)=NULL
sbp_se=unlist(sbp_koges_se)
names(sbp_koges_se)=NULL
fbs_be=unlist(fbs_koges_be)
names(fbs_koges_be)=NULL
fbs_se=unlist(fbs_koges_se)
names(fbs_koges_se)=NULL


MRdata_sbp_fbs<- mr_input(bx = sbp_be, bxse = sbp_se, by = fbs_be, byse = fbs_se)
mr_allmethods(MRdata_sbp_fbs, method ="main")
mr_plot(mr_allmethods(MRdata_sbp_fbs, method = "main"))
mr_plot(MRdata_sbp_fbs, orientate = TRUE, line = "egger")
```

국 문 요 약 (Korean Abstract)

생애주기 접근법 기반
당뇨병과 고혈압의 양방향 인과관계

연세대학교 일반대학원 보건학과

전 주은

연구배경: 기존의 많은 대규모 역학 연구들에서 제2형 당뇨병과 고혈압 사이에 정적인 관계가 있음이 입증되어왔다. 그러나 기존의 분석 방법은 대부분 제2형 당뇨병과 고혈압간의 양방향이 아닌 한 방향 관련성을 전제로 하고 있었다. 기존 관찰 연구들에서는 교란 요인과 역 인과 관계에 의해 도입된 잠재적 편향으로 인해 인과 추론에 제한적이었으며, 멘델리안 무작위 시험(Mendelian randomization, MR) 연구방법 또한 측정되지않은 잠재 교란변수나 유전학적 변수 특유의 다면발현성으로 인해 명확한 인과관계 도출에는 한계가 있었다. 따라서 양방향 관련성 연구를 위한 보다 개선된 연구방법론이 필요한 실정이다. 최근 유전학적 기반의 MR 연구방법을 통해 영국 UK-바이오뱅크 대상자에서 제2형 당뇨병과 고혈압간의 양방향 인과관계를 탐구한 단 하나의 선행연구가 있으나, 서양인들만을 대상으로 한 결과라는 일반화 한계점이 있다.

연구목적: 이에 이 연구는 선행 연구방법을 개선하여 MR 연구방법론 중 5가지 방법론 비교를 통해 한국인에서 공복혈당(FBS)과 수축기혈압(SBP) 수준의 양방향 인과 관계를 규명하고, 생애 과정 접근을 기반으로 이 인과관계를 보다 확장하기 위해 잠재계층궤적분석을 이용하여 유전자 기반으로 추정된 FBS와 SBP 수준의 시변(Time-varying changes)을 파악하여 이에 따른 후차적인 당뇨병과 고혈압 발생 위험도를 평가하고자 하였다.

연구방법: 한국인 대상 대규모 Biobank(KCPS-II)에서의 전장유전체연관분석(GWAS)을 통해 추출된 SNPs를 Instrumented genetic variants로 이용하여 Two-sample MR 연구방법을 통한 FBS와 SBP 수준의 양방향 인과관계를 규명하였다. 양방향 인과 관계 평가에는 2 단계 최소자승 (2 stage least squares, 2sls)방법, 역분산가중 (inverse-variance weighted, IVW) 방법, 2개의 중앙

값 기반 방법(Simple median and weighted median) 및 MR-Egger 방법을 포함한 5가지 MR 방법이 적용되었다. 이 후, 연구기반시점 기준 당뇨병, 고혈압 및 뇌졸중이 없는 건강한 일반인을 대상으로 선형 회귀분석에 의해 유전자 기반 FBS와 SBP 수준의 추정치를 도출하였으며, 잠재계층궤적분석을 시행하여 유전자 기반 FBS와 SBP 추정치의 시변을 파악하고 그에 따른 후차적인 당뇨병과 고혈압 발생 위험도를 각각 평가하였다.

연구결과: 우리나라 대규모 Biobank(KCPS-II)의 GWAS 결과에서 p-vlaue < $1.0x10^{-8}$ 기준을 만족하는 91개(FBS) 및 68개(SBP) 단일 염기 다형성(SNP)이 한국인전장유전체연구자료(KoGES)에 부합되어 최종적으로 선정되었다. 멘델리안 무작위 시험 연구결과, 2SLS방법 하, 유전적 변이로 인한 FBS10mg/dL 상승은 SBP 1.63mm/Hg(p=0.005)의 증가와 관련이 있었고 유전적으로 결정된 SBP의 10mm/Hg 상승은 FBS 11.39mg/dL의 증가와 관련이 있었다(p<.0001). MR-egger 방법에서 유전적 변이로 인한 FBS 1mg/dL 상승은 SBP 0.20mm/Hg (p<.0001)의 증가와 관련이 있었고 유전적으로 결정된 SBP의 1mm/Hg 상승은 FBS 1.08mg/dL의 증가와 관련이 있었다(p<.0001). 또한, 연구기반시점 기준으로 당뇨병, 고혈압 및 뇌졸중 과거력이 없는 건강한 일반인을 대상으로 선형 회귀분석에 의해 유전자 기반 FBS와 SBP 수준의 추정치를 도출하였으며, 잠재계층궤적분석을 통해 시간 경과에 따른 유전자 기반 FBS와 SBP 추정치의 변화를 파악하였다. 구현된 시간 경과에 따른 유전자 기반 FBS와 SBP 추정치의 패턴에 따른 후차적인 고혈압 및 당뇨병 발병 위험도를 추가로 확인하였다. 특히 추적기간동안 수축기혈압(SBP) 수준 변동성이 많았던 “Uncontrolled”군에서 꾸준히 정상치를 유지한 “Controlled”군에 비해 성별, 연령, 체질량지수(BMI), 흡연력, 음주력, 운동 및 고혈압약 복용력 모두 통제하였을 때, 후천적인 당뇨병 발생 위험이 20% 높았다.

결론: FBS와 SBP 사이의 양방향 인과 관계는 생애 과정 접근법을 기반으로 보다 구체화되었다. 생애주기 기반으로 한 FBS와 SBP 사이의 양방향에 대한 증거를 추가하기 위해서는 더많은 유전적 변이와 대사성 바이오마커를 포함하는 보다 더 정교한 대규모 바이오뱅크 연구가 필요할 것이다.

핵심어: 전장유전체연관분석, 단일염기다형성, 멘델리안 무작위시험, 제2형 당뇨병, 고혈압