Triangulation study on the causal effect of fasting serum glucose on atherosclerotic cardiovascular disease

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ABSTRACT

Triangulation study on the causal effect of fasting serum glucose on atherosclerotic cardiovascular disease

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Background: Diabetes is a known risk factor that increases the risk of atherosclerotic cardiovascular disease (ASCVD), but previous observational studies have not been able to establish a causal relationship between dysglycemia-related markers and the incidence of cardiovascular disease. However, in recent epidemiological research, triangulation has been proposed as a scientific research method that can improve the limitations of causal inference. The purpose of this study is to confirm the causal relationship between fasting serum glucose (FSG) and ASCVD using triangulation.

Methods: In this study, triangulation was analyzed using meta-analysis of clinical trials, cohort analysis, and Mendelian randomization (MR) analysis. The meta-analysis was performed on clinical trials (RCTs) and observational cohort studies, respectively. The cohort analysis used data from the Korean Cancer Prevention Study(KCPS)-II. KCPS-II subjects were 159,844 people who visited 18 comprehensive health checkup centers across the country, including Seoul and Gyeonggi Province, from 2004 to 2013. A blood-based prospective cohort study was established by obtaining consent forms for research participation. am. Mendelian randomization (MR) analysis used data from the Korean Genome Epidemiology Study (KOGES) biobank for fasting blood sugar, and data confirmed in the Korean cancer

prevention study was used to determine the occurrence of atherosclerotic cardiovascular disease. Additionally, the Biobank of Japan (BBJ) was also used for atherosclerotic cardiovascular disease data. Mendelian randomization (MR) analysis was performed using standard (one-sample) MR within the Korean Cancer Prevention Study-II, two-sample MR using KOGES and KCPS-II biobanks, and genetic variables of systolic blood pressure and LDL cholesterol. Controlled multivariate MR (MVMR) was analyzed separately. In this study, fasting blood sugar was affected by every 10 mg/dL increase.

Results: All studies have shown that FSG has an effect on ASCVD. First, in a meta-analysis using cohort studies, the risk of ASCVD increased by 3.7% for every 10 mg/dL increase in fasting blood sugar (HR=1.037, 95% CI=1.022-1.053). Second, a cohort study estimated that for each 10 mg/dL increase in FSG level, the risk of ASCVD increased by 4.9% (HR=1.049, 95% CI=1.039-1.059). Third, the results of the standard MR study also showed that for evert 10 mg/dL increase in FSG influenced the risk of ASCVD (HR=1.134, 95%CI=1.038-1.238). Fourth, the results of the two-sample MR study also showed that for evert 10 mg/dL increase in FSG influenced the risk of ASCVD (HR=1.110, 95%CI=1.040-1.180). Lastly, the results of the multivariable MR study showed that the OR per 10 mg/dL increase in FSG

in ASCVD was 1.138, which was statistically significant (95% CI=1.068-1.214, p-value=<0.001). This study showed similar results for a 10 mg/dL increase in FSG and ischemic heart disease, but did not show a significant relationship with stroke.

Conclusion: FSG has been shown to have a causal relationship with the occurrence of ASCVD in Koreans. Therefore, the results of this study suggest that glycemic control has the potential to substantially reduce the lifetime risk of cardiovascular disease.

Keywords: Fasting serum glucose, atherosclerotic cardiovascular disease, Triangulation

Ⅰ. INTRODIUCTION

1. Study background

Diabetes is a known risk factor that increases the risk of atherosclerotic cardiovascular disease (ASCVD). Previous meta-analyses and observational studies supported the association between dysglycemia-related parameters (e.g. fasting glucose, HbA1C and diabetes) with Stroke [1] and cardiovascular disease[2-6], but causality could not be established due to confounding and reverse causality. Randomized clinical trials (RCTs) allow reliable causal inferences, but recent RCTs have confirmed that lowering blood glucose levels does not prevent cardiovascular disease[7,8]. These results show uncertainty about the causal relationship between diabetes and cardiovascular diseases such as coronary artery disease (CAD). Furthermore, there are some uncertainties about the relationship between subthreshold blood glucose levels and cardiovascular disease risks for diagnosing diabetes, such as the emerging U-shape between blood glucose and cardiovascular disease risk[4, 5, 9].

However, in recent epidemiological research, triangulation has been proposed as a scientific research method that can improve the limitations of

causal inference[10]. There are four types of triangulation. The first is data triangulation, which involves using the data differently, the second is researcher triangulation, which consists of using multiple observers rather than a single observer on the same object, and the third is theoretical triangulation, which consists of using more perspectives rather than a single perspective on the same object. The fourth is methodological triangulation, which uses different methods for the same theory. The triangulation used in this dissertation is data triangulation and methodological triangulation[11]. Research results may be biased depending on how researchers design the study when testing a research hypothesis, and these research results may raise issues in terms of reliability and validity. Therefore, if the directions of the results of the various analysis methods included in the triangular research method are all consistent, the validity and reliability of the hypothesis to be confirmed can be considered high. The triangulation method in epidemiological research to identify causes is defined as “a method of integrating the results of multiple analysis methods with different potential biases and using these differences to infer causality.”[12]

In this study, triangulation is analyzed using Meta-analysis, MR, and Cohort study. The main cause of bias in observational studies is unmeasured

or incorrectly measured confounding variables. Bias in meta-analysis can occur due to publication bias and selection bias. Lastly, MR is unlikely to be biased by characteristics such as socioeconomics and lifestyle, but bias can occur due to population stratification, etc. As such, the causes of bias that occur in each of the three research methods are different. Therefore, scientific verification is required to reveal the causal relationship of the research hypothesis by integrating the results derived from different approaches.

2. Objectives

To investigate whether fasting blood glucose levels have a causal relationship with ASCVD risk in the Korean population, the objective of this study are as follows:

(1) Investigate causality through meta-analysis and cohort studies on fasting serum glucose and cardiovascular disease.

(2) Analyze the relationship between fasting serum glucose and cardiovascular disease through a cohort study.

(3) Investigate the causal relationship between fasting serum glucose and

cardiovascular disease using the Mendelian randomization method.

Finally, causality between fasting serum glucose and cardiovascular disease is inferred through a triangulation approach.

Ⅱ. MATERIALS AND METHODS

1. Study population and data sources

1-1. Korean Cancer Prevention Study (KCPS)-II

The data used in this study are from the Korean Cancer Prevention Study (KCPS)-II biobank, which were collected from 160,407 subjects who visited a total of 18 comprehensive examination centers, including 15 centers in Seoul and Gyeonggi Province and 3 centers in other regions, from 2004 to 2013 for the purpose of examination [13-15]. From 2004 to 2013, there were all individuals who provided informed consent for the study among the health examination participants.

Within this population, 159,844 people with genetic information were available for the study. After excluding those with missing or extremely abnormal values for the essential variables, including diabetes mellitus, those with a history of ASCVD, and those on FSG-lowering medication, the final 152,071 participants were satisfied.

At baseline, all participants were asked to describe their weight and height. Blood was collected while fasting for more than 8 hours, and

triglyceride (TG), gamma-glutamyl transferase (GGT), fasting blood glucose (FBG), hemoglobin A1c (HbA1c), and insulin were measured. HOMA-IR was calculated as fasting insulin concentration (mU/L) × fasting blood glucose concentration (mmol/L)/22.5 [16]. FSG levels were measured using COBAS INTEGRA 800 and Hitachi-7600 analyzers (Hitachi, Tokyo, Japan), respectively.

We defined incident ASCVD as the first hospital admission with an ASCVD diagnosis (ICD-10 codes), or death with ASCVD as the underlying cause if there was no relevant hospital admission (ICD-10 codes)[17]. This study's detailed outcomes include ischemic heart disease (IHD) (ICD-10 codes I20-I25), stroke (ICD-10 codes I60-I69), and other CVD (ICD-10 I10-I15, I70-I74, R96, I50, I51, I44-I49). IHD was classified into two types: angina pectoris and acute myocardial infarction. Strokes were classified with either ischemic or hemorrhagic.

1-2. Korean Genome and Epidemiology Study (KoGES)

KoGES, a large-scale prospective genomic and epidemiological cohort study, provides long-term follow-up of common complex diseases (Type 2 Diabetes mellitus, hypertension, obesity, metabolic syndrome, osteoporosis, cardiovascular disease, cancer) and causes of

death in Koreans. Genetic factors for fasting serum glucose were discovered in a total of 72,299 people. The average age of the subjects included in the study was 54.65 years old, and the average fasting serum glucose level was 93.72 mg/dl [18].

1-3. BioBank Japan (BBJ)

In 2003, BioBank Japan (BBJ) started developing one of the world's largest disease biobanks, creating a foundation for research aimed at achieving medical care tailored to the individual traits of each patient. No less than 5,800 items of screened information are available for research, including the patients' survival information, with 95% of the patients tracked over an average of 10 years [19]. Blood sugar-related genetic variants analyzed in 45,383 Type 2 diabetes subjects and 132,032 controls were used as exposure-related instrumental variables. In GWAS (Genome Wide Association Study), a large number of diabetes-related genes KCNQ1, PAX4, DUSP9, CDKAL1, TCF7L2, IGF2BP2, HHEX, CDC123, and SLC30A8 were discovered.

1-4. MEGASTROKE consortium

The MEGASTROKE consortium, a large-scale international collaboration launched by the International Stroke Genetics

Consortium, releases the summary statistics from the 2018 meta-analysis of Genome-wide Association (GWA) data in stroke and stroke subtypes to enable other researchers to explore these data for scientific purposes[20]. We provide two meta analysis results, and we use the trans-ethnic results. There are a total of 5 types of strokes. (1) any stroke = AS, (2) any ischemic stroke = AIS, (3) large artery stroke = LAS, (4) cardioembolic stroke = CES, (5) small vessel stroke = SVS.

2. Genotyping procedures and GWAS

In order to conduct MR, all subjects need genetic test data. 50% of the subjects in this study had a global screening array (GSA) chip tested. The remaining 50% took the Korea Biobank Array [21]. Then, based on 1,000 genomes in the identical way, each imputation was performed using IMPUTE 5 to build integrated data. IMPUTE 5 is a software program designed for imputing and estimating unobserved and missing genotype data of individuals using known haplotype panels and recombination maps. After the quality control criteria used for GWAS analysis (minor allele frequency (MAF) 0.01, Hardy-Weinberg Equilibrium (HWE)$<10^{-6}$, 6,804,815 single nucleotide polymorphisms (SNPs) were used for analysis. In order to estimate the effect size of each SNP for triglyceride (TG), gamma-glutamyl transferase (GGT), body mass index (BMI), FSG, HbA1c, and Insulin resistance (HOMA-IR), linear regression including gender, age, chip type, and principal component (PC) was used. In this study, GWAS analysis was performed using PLINK 2.0.

3. Statistical methods

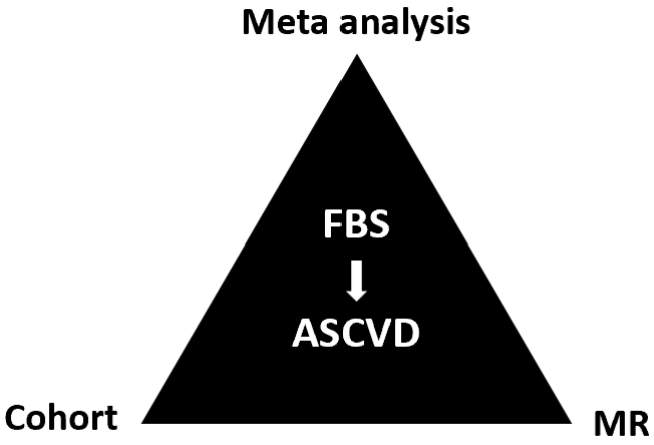

Figure 1. Triangulation design

There are limitations to conducting scientific research on populations or people. Hypotheses that evaluate the risk of hazardous substances cannot be designed in experimental studies on humans due to ethical issues. However, in recent epidemiological research, triangulation has been proposed as a scientific research method that can improve the limitations of causal inference.

The triangulation method has been used since the 1600s to calculate the distance between two objects that are difficult or impossible to measure, using mathematical characteristics. Recently, it has been used to compare

research results using two or more different methodologies in qualitative analysis that is difficult to determine. Looking for an answer to a question. Research results may be biased depending on how researchers design the study when testing a research hypothesis, and these research results may raise issues in terms of reliability and validity. Therefore, if the directions of the results of the various analysis methods included in the triangular research method are all consistent, the validity and reliability of the hypothesis to be confirmed can be considered high. Conversely, if there is heterogeneity in the results of various analysis methods included in the triangular research method, causal questions can be resolved or new hypotheses can be proposed by confirming the direction of the analysis methods with heterogeneity. The triangulation method in epidemiological research to identify causes is defined as "a method of integrating the results of multiple analysis methods with different potential biases and using these differences to infer causality."

In epidemiological studies, the triangulation method must meet the following criteria:

1. The results of at least two different research methods with different potential biases should be compared.
2. The multiple research methods used in triangulation have different

approaches but must test the same hypothesis.

3. When comparing results, the time when the results were derived from each research method and the period of exposure to risk factors should be considered.

4. When comparing the results of each research method, the cause of bias should be identified.

In epidemiological research, the research methods that constitute the triangular research method that satisfies the above four criteria can be meta-analysis, Cox regression analysis of prospective cohort studies, and MR. The main cause of bias in observational studies is unmeasured or incorrectly measured confounding variables. Bias in meta-analysis can occur due to publication bias and selection bias. Lastly, MR is unlikely to be biased by characteristics such as socioeconomics and lifestyle, but bias can occur due to population stratification, etc. As such, the causes of bias that occur in each of the three research methods are different. Therefore, scientific verification is required to reveal the causal relationship of the research hypothesis by integrating the results derived from different approaches.

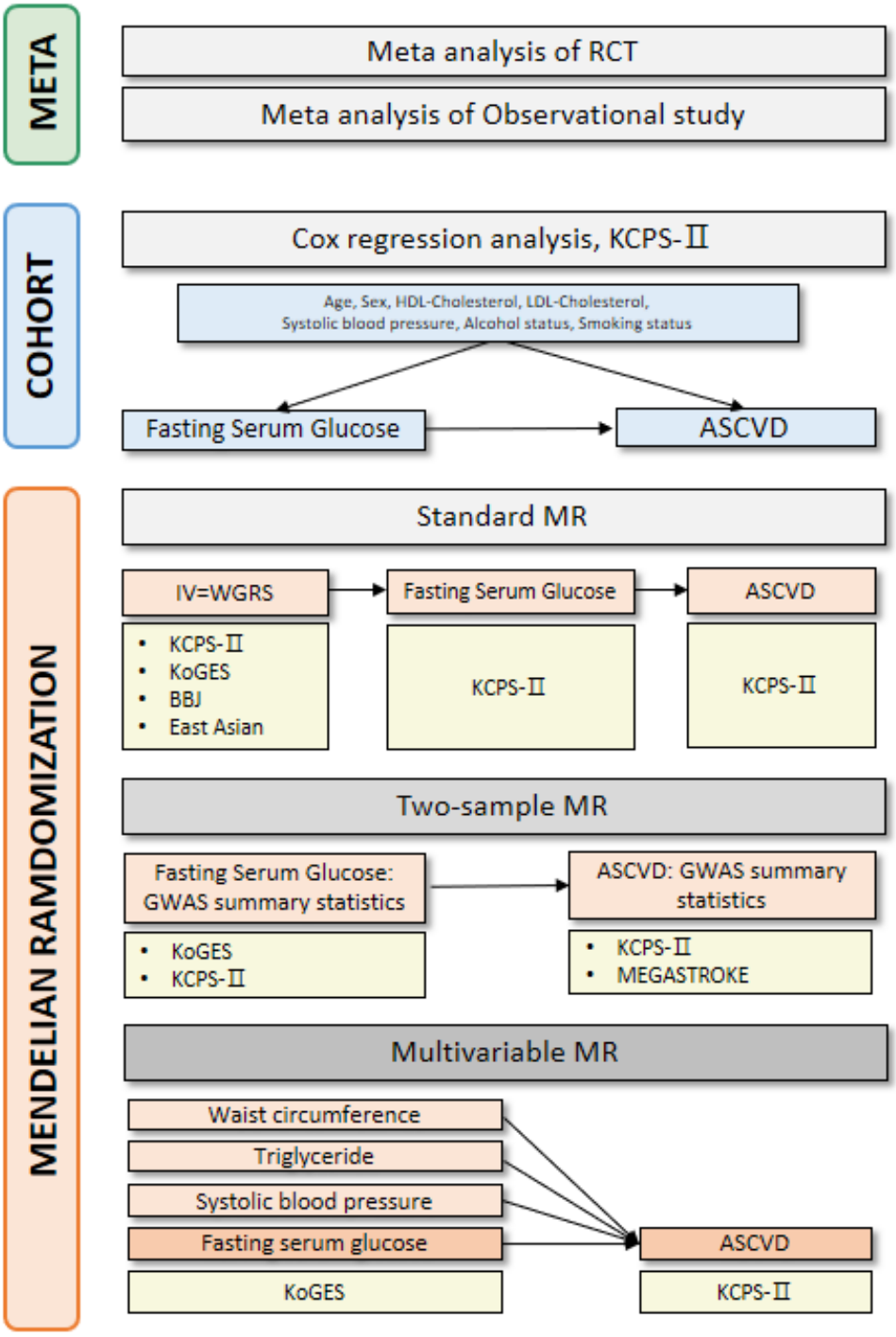

Figure 2. Conceptual framework in research

2-1. Meta analysis

2-1-1. Meta analysis of RCT

2-1-1-1. Literature search

This systematic review was conducted in adherence with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [22].

Our meta-review searched for meta-analyses of RCT studies on the association between FSG and CVD. CVD is a general term for many cardiovascular and cerebrovascular diseases, such as coronary artery disease (CAD), heart failure (HF), and stroke [23]. CAD is the most common type of heart disease, also known as coronary heart disease (CHD) or ischemic heart disease, and mainly includes stable angina, unstable angina, myocardial infarction (MI), or sudden cardiac death [24]. Stroke refers to fatal and non-fatal ischemic and hemorrhagic strokes, with no emphasis on initial or recurrent events. We included studies that investigated the association between FSG and all-cause and cardiovascular mortality. We searched for articles published in English before October 8, 2023 in two databases: PubMed and Web of Science. Search terms include: (“fasting blood glucose” or “fasting serum glucose” or “fasting serum glucose” or “fasting serum sugar” or “diabetes” or “prediabetes”) and (“cardiovascular

disease" or "coronary artery disease" or "coronary heart disease" or "angina" or "myocardial infarction" or "heart failure" or "stroke") and ("random" or "clinical" or "trials" or "intervention"). Additionally, the references of the articles were further reviewed to retrieve relevant articles.

2-1-1-2. Eligibility criteria

Human studies were considered eligible for inclusion if they satisfied the following predefined criteria: (1) RCTs were included. (2) Patients with cardiovascular disease were included. (3) Diabetes and/or prediabetes assessment scales were assessed before and after the intervention. (4) Published as a full-text article in a peer-reviewed scientific journal. Studies were excluded if (1) they included patients with other diseases or only healthy controls (HC) and (2) they were published as reviews, case reports, conference abstracts, or letters.

2-1-1-3. Data extraction

Data extracted from RCTs were first author, year of publication, sex (%female), mean age range of participants, BMI, CVD history, intervention, control, outcome, intensive intervention duration, total follow-up duration, n of case, n of control, n of intervention group, n of control group, and definition of diabetes.

2-1-1-4. Data analysis

RR values from MR studies requiring analysis were pooled using a random effects model, and pooled results were evaluated using forest plots. Heterogeneity across studies was assessed using the I^2 statistic and was considered mild if I^2 was between 25% and 50%, moderate if I^2 was between 50% and 75%, and severe if I^2 was greater than 75%. A two-sided P-value of <0.05 was considered statistically significant.

2-1-2. Meta analysis of cohort study

2-1-1-1. Literature search

This study was designed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [22] and the Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines [25]. The Participant, Intervention, Comparison, Outcome (PICO) [26] strategy was used to establish the guiding question of this review (Santos et al., 2007). The PICO characteristics were (1) general population (2) fasting blood glucose (3) compared with a control group (4) cardiovascular disease.

PubMed and MEDLINE were searched from inception to 26 October 2023. The search strategy included keywords and MeSH terms relating to fasting serum glucose and cardiovascular disease. The searching strategy for the

PubMed database was as follows: ("fasting blood glucose" or "fasting serum glucose" or "fasting serum glucose" or "fasting serum sugar") and ("cardiovascular disease" or "cardiovascular disease" or "coronary artery disease" or "coronary heart disease" or "stroke") and ("cohort"). We excluded studies published as abstracts. The review was restricted to original articles published in English. We also manually searched bibliographies of included studies as well as existing systematic reviews for any other articles that may be potentially suitable.

2-1-1-2. Eligibility criteria

Human studies were considered eligible for inclusion if they satisfied the following predefined criteria: (1) they were non-RCTs, open trials, cross-sectional trials, or prospective trials; (2) Patients with cardiovascular disease were included. (3) the risk estimate was reported as an odds ratio (OR), hazard ratio (HR) or relative risk (RR); (4) the 95% CI for the risk estimate was included (5) Published as a full-text article in a peer-reviewed scientific journal. Studies were excluded if (1) they included patients with other diseases or only healthy controls (HC) and (2) they were published as reviews, case reports, conference abstracts, or letters.

2-1-1-3. Data extraction

Data extracted from cohort studies were first author, year of publication, mean age range of participants, median follow-up time, n of case, n of control, and definition of CVD.

2-1-1-4. Assessment of study quality

Five authors independently scanned all titles and abstracts and excluded articles that clearly were not observational studies on the topic. We proceeded to assess full-text versions of potentially relevant articles and conducted more detailed checks against our eligibility criteria. Disagreements were resolved by discussion.

2-1-1-5. Data synthesis

The standard unit is set at 10mg/dL, and the formula for changing the effect size when converting 1mg/dl to 10mg/dL is as follows.

- $1\text{mg/dL} \rightarrow \alpha \times \text{mg/dL} = effect\ size \rightarrow effect\ size^{\frac{\alpha}{1}}$

The formula for changing the effect size when converting 1 mmol/L to 10mg/dL is as follows.

- $1\text{mg/dL} = 18.0182 \times 1\text{mmol/L}$ [27]

- $1\text{mmol/L} \rightarrow \frac{1}{18.0182} mmol/L = \alpha \times \text{mmol/L} \rightarrow \frac{\alpha}{18.0182} mmol/L$

- $1\text{mmol/L} \rightarrow \alpha \times \text{mg/dL} = effect\ size \rightarrow effect\ size^{\frac{\alpha \times 18.0182}{1}}$

2-1-1-6. Data analysis

A random-effects model was utilized because it was assumed that the variation in the actual effect size follows a normal distribution, and the heterogeneity observed within and among the studies was attributed to unanticipated factors rather than remaining effects[28, 29]. We examined the heterogeneity using the I^2 statistic, assuming a value of 25% to indicate low heterogeneity, 50% as moderate, and 75% as high. A two-sided P-value of <0.05 was considered statistically significant.

2-2. Observational study (Cohort)

ASCVD participants with and without were compared in terms of socio-demographics, behavior, medical history, and ASCVD risk factors. We examined the associations between FSG and Strokes, IHD, and MI separately. After confirming that the proportionality assumption was met, we created unadjusted and adjusted Cox proportional hazards models. We first adjusted for baseline age, sex, and then for HDL-Cholesterol, LDL-Cholesterol, Systolic blood pressure, Alcohol status, and Smoking status.

SAS v 9.4 (Cary, NC) was used for statistical analyses. All statistical tests were two-sided and $p<0.05$ was considered statistically significant.

2-3. Mendelian Randomization

2-3-1. Standard MR

2-3-1-1. Statistical power calculation

We estimated statistical power for our MR analysis by an online web tool (https://sb452.shinyapps.io/power/) [30]. Statistical power for MR given a specific sample size based on several parameters, including the proportion of variants (R^2) in the exposure explained by genetic instruments; the causal effect of the exposure on the outcome, and the ratio of cases to controls (for binary outcome). Using the KCPS-Ⅱ GWAS sample size (n = 159,844) and the ratio of cases to controls (1 to 1.908), we calculated statistical power for our MR analysis. We had 83.4% power to detect the relationship between FSG and ASCVD. For MI, there might be not sufficient power (45.3%). For 2 SNPs on walking, we calculated sufficient power (100%) to detect the effects of digital-device walking on AD. For 25 genetic variants on moderate-intensity behavior, we calculated sufficient power

(97.7%) to detect the effects of moderate-intensity activity on AD.

2-3-1-2. Polygenic Risk Score (PRS)

Based on the relationship between the SNPs and fasting glucose levels, we assumed an additive genetic model with scores of 0, 1, or 2 for genotypes containing 0, 1, or 2 alleles, respectively. PRS was calculated by adding the scores for each SNP and multiplying them by the β from another population study[31]. We calculated PRS using the KoGES GWAS results, the GWAS results from the Japanese BBJ data and the SNP from a previous paper on the East Asian population. The PRS used in our study was calculated in plink1.9. The PRS calculation formula is as follows $PRS_j = \frac{\sum_i^N S_i * G_{ij}}{P * M_j}$, where the effect size of SNP i is S_i; the number of effect alleles observed in sample j is G_{ij}; the ploidy of the sample is P (is generally 2 for humans); the total number of SNPs included in the PRS is N; and the number of non-missing SNPs observed in sample J is M_j.

A sufficiently large dataset is required for a standard-sample approach, which includes enough data to assess all three stages: the genotypes of the genetic instruments, the exposure, and the outcome. For the two-stage least squares method (2SLS) [32], the exposure is regressed on the genetic instruments and fitted values, also called genetically determined values, of

the phenotype, are calculated (first stage). The genetically determined biomarker is then regressed on the outcome/disease (second stage), yielding a single estimate of the causal effect. The Cragg-Donald F-statistic calculation formula is as follows $F = \frac{\left(R^2 * (n-2)\right)}{(1-R^2}$, used to estimate the strength of the association, and F values > 10 were regarded as useful for MR analysis[33].

2-3-2. Two-sample MR

2-3-2-1. Inverse variance weighted method

The most popular method is the inverse-variance weighted (IVW) method, which uses multiple variants as instruments[34]. An IVW meta-analysis of the ratio estimates for the individual variants can be used to determine the IVW estimate for uncorrelated variants[35]. Using a weighted genetic risk score as a single instrument and weights equal to the associations of each variant with the exposure estimated in the first sample, the same estimate can be calculated equivalently as the ratio estimate[36]. To accommodate correlation (linkage disequilibrium) between variants, this method has been modified. The 2SLS estimate derived from individual level data is asymptotically equivalent to the IVW estimate for continuous outcomes. The most effective estimate of is produced by the 2SLS method,

which is also the IVW method.

2-3-2-2. MR Egger

The MR-Egger method calculates the average pleiotropic effect as the intercept and the slope from the weighted regression of the variant—outcome associations on the variant—exposure associations, respectively. The approach permits pleiotropic effects from any genetic variant, but it stipulates that these effects must be independent of variant-exposure associations (known as the Instrument Strength Independent of Direct Effect (InSIDE assumption))[37]. If there is "correlated pleiotropy," which happens when genetic variations affect an exposure and outcome confounder, this supposition would be broken [38].

2-3-2-3. Weighted median

Weighted median methods assume that more variants estimate the true causal effect than estimate other quantities, assuming that less than half of the variants are invalid instruments (the majority validity assumption)[39]. It is robust against outliers and sensitive to adding and removing instrumental variables.

2-3-2-4. MR-PRESSO

MR-PRESSO evaluates pleiotropy from a different perspective. MR-PRESSO adopts a “leave-one-out” approach to evaluate whether specific SNP instrumental variables lead to differences in the calculated RSS from what is expected from the simulation[40, 41]. Simply put, the model involves three steps, determining the magnitude of horizontal pleiotropy.

2-3-3. Multivariable MR(MVMR)

In The causal effect of an exposure on an outcome of interest, including effects through potential mediators, is the "total" effect of the exposure on the outcome. Total effect = direct effect + indirect effect. The proportion of the total effect mediated from these effects can be calculated as "indirect effect/total effect." Mediation analysis uses regression analysis to distinguish between direct and indirect effects of an exposure on an outcome and calculate the mediated proportion[42, 43].

MVMR is an extension of MR that allows for the causal effects of multiple exposures on the outcome to be estimated. MVMR estimates the "direct" causal effect of each exposure included in the estimate on the outcome,

conditional on the other exposures included in the model. It is therefore particularly useful when two or more potentially related exposures are of interest and the researcher wishes to understand whether both exposures have a causal effect on the outcome or, as discussed later, whether one exposure is potentially a mediator of the other. MVMR requires a set of SNPs that are associated with exposure variables but do not affect the outcome except through these variables. In the same way as standard MR, these SNPs are used to predict each exposure variable in the model, and these predicted values are used in multivariate regression analyzes to estimate the effect of the exposure on the outcome. For individual-level data, MVMR is implemented through 2SLS regression of the model[44].

For statistical analysis in this study, R version 4.1.2 (R Development Core Team,Vienna, Austria) and SAS version 9.4(Cary, NC) were used. All of the analyses were calculated using R packages ('Guido-s/Meta', 'Therneau/Survival', 'Kassambara/Survminer', 'Zeileis/Ivreg', 'MRCIEU/TwoSampleMR'[14], 'WSPILLER/MVMR'[38], 'Gforge/Forestplot[3.0]', 'Rondolab/MR-PRESSO')

This study protocol was reviewed and approved by the Institutional Review Board (IRB) of Severance Hospital (Seoul, Korea) and the informed consent was received (IRB No: 4-2011-0277).

Ⅲ. RESULTS

PART 1. Meta analysis

1. Literature search and study selection of RCTs

A total of 2,795 articles were searched in the two databases, of which 1,333 articles had no duplicates. After the initial screening of article titles and abstracts, 68 articles were considered relevant to this meta-review. Finally, 5 RCT studies were included (Figure 3, Table 1).

The characteristics of the RCT studies included in the meta-analysis are in Table 1. As described in Table 1, five parallel RCTs were included, resulting in a total of 11,017 subjects. Three studies included patients with prediabetes[45-57] and two with diabetes[47, 48]. The average intervention period was 5.2 years, and the total follow-up period was 2 to 30 years. In all trials, lifestyle interventions combined diet and physical exercise recommendations. Regarding pharmacological treatment, only one study used metformin as one treatment arm and no other drugs that could have influenced the study results[46].

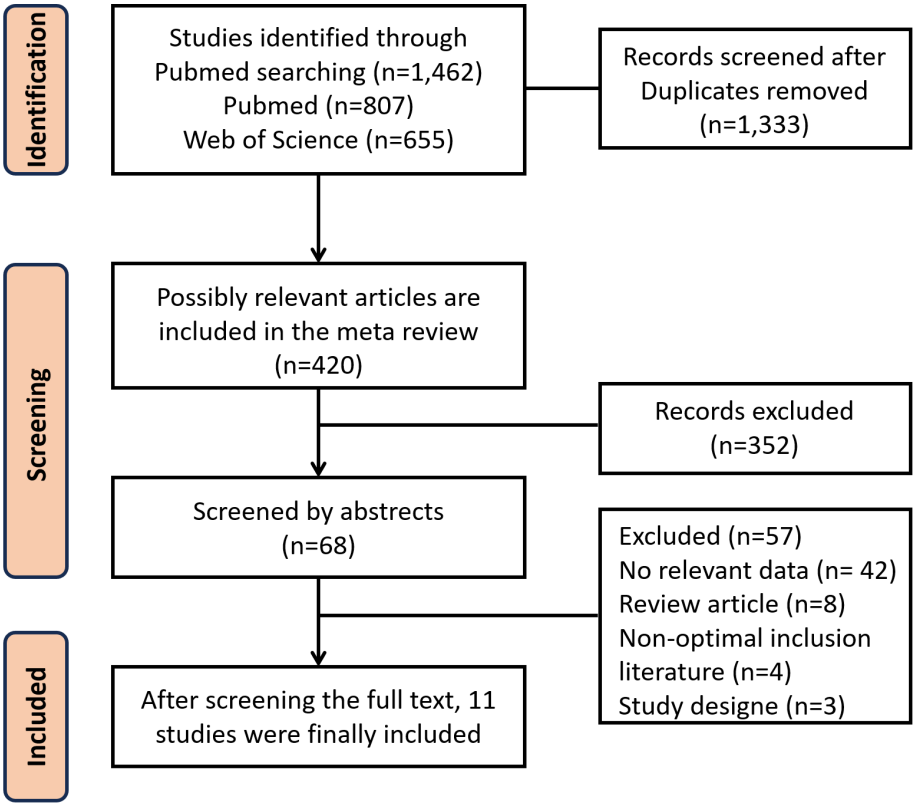

Figure 3. Assessment of the quality of the included RCTs

Table 1. Diabetes and cardiovascular diseases in RCTs meta-analyses

Study	Population characteristics	Intervention vs. control	Outcome(s)	Intensive intervention duration	Total follow-up duration
Oldroyd et al., 2006 [44]	Prediabetes, 43.5% female, age 57.9 ± 0.3 years, BMI NI, previous CVD NI	Dietary and exercise prescription vs. no advice about lifestyle	CV and all-cause mortality	2 years	2 years
Griffin et al., 2019 [47]	T2D, 42.1% female, age 60.3 ± 6.9 years, BMI 31.6 ± 5.6 kg/m^2, history of myocardial infarction in 6.1% and stroke in 2.2%	Small group-based activities vs. usual care according to each center	CV and all-cause mortality	5 years	10 years
Gong et al., 2019 [45]	Prediabetes, 45.8% female, age 45.2 ± 9.3 years, BMI 25.7 ± 7.6 kg/m^2, previous CVD NI	Dietary and exercise prescription vs. brochures about lifestyle but no specific advice	CV and all-cause mortality	6 years	30 years
Lee et al., 2021 [46]	Prediabetes, 68.5% female, age 50.5 ± 11 years, BMI 34 ± 6.7 kg/m^2, 29% with hypertension and 69% hyperlipidemia	Dietary and exercise prescription vs. placebo + standard advice	CV and all-cause mortality	3 years	21 years
Look AHEAD Research Group, 2022 [48]	T2D, 59.5% female, age 58.8 ± 6.9 years, BMI 35.9 ± 5.9 kg/m^2, 14% with history of CVD	Dietary and exercise prescription vs. three group sessions about lifestyle	CV and all-cause mortality	10 years	16.7 years

CV, cardiovascular; CVD, cardiovascular disease; NI, not informed; T2D, type 2 diabetes; Data are presented as mean ± SD.

2. Association between Diabetes and cardiovascular diseases from Meta analysis of RCTs

A total of 5 RCT studies were included in this study (Table 1). The results of meta-analysis on Prediabetes and Type 2 Diabetes and CVD in RCT studies are as follows (Figure 3). The cardiovascular mortality analysis included 11,017 subjects (51.9% female, mean age 54.5 years, mean BMI 31.8 kg/m^2), with an overall outcome incidence rate of 5.69%.

MR results from an RCT study showed that the risk of CVD was 17% lower in the intervention group compared to the diabetes group, and heterogeneity was very low at 3%. (RR=0.83; 95% CI=0.72–0.96; I^2=3%).

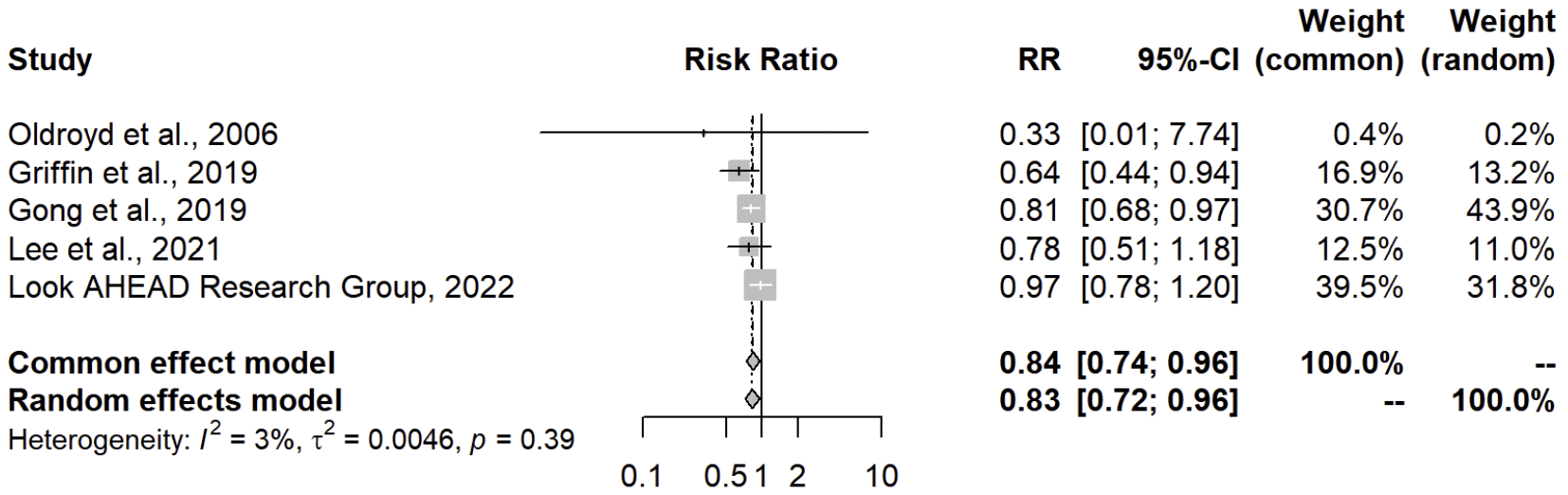

Figure 4. Meta-analysis results for the association between Fasting blood glucose and CVDs in RCT studies

3. Literature search and study selection of cohort studies

A total of 2,050 articles were searched in the two databases, of which 2,045 articles had no duplicates. After the initial screening of article titles and abstracts, 921 articles were considered relevant to this meta-review. Finally, 8 cohort studies were included (Figure 5).

The characteristics of the cohort studies included in the meta-analysis are in Table 2. Each cohort study presented the comparative risk of developing ASCVD per 1 mg/dl increase or 1 mmol/L as a categorized variable. In this study, the unit was unified as mg/dL. And the Generalized least squares for trend estimation (GLIST) method was used to estimate the relationship per 10 mg/dL increase. First, to obtain the comparative risk per 1 mg/dl increase, the comparative risk (95% confidence interval), median fasting blood sugar, and sample number were calculated for the reference group and the group with the highest comparative risk, respectively, and then calculated using the GLIST method.

At this time, in the case of papers reporting mmol/L, fasting blood sugar was multiplied by 18.02 to convert to mg/dL. Additionally, the regression coefficient was finally multiplied by 10 to obtain the comparative risk (95% confidence interval) per 10 mg/dL increase in fasting blood sugar.

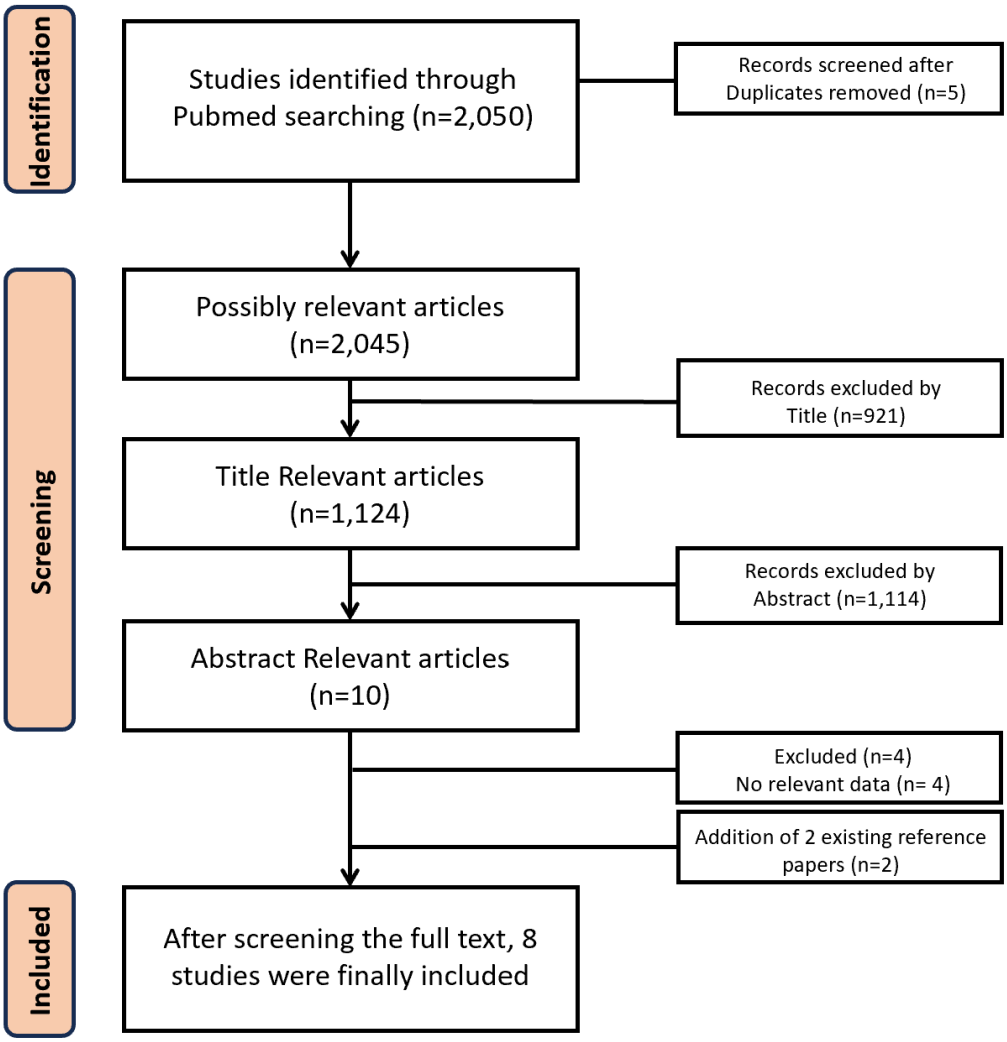

Figure 5. Assessment of the quality of the included cohort studies

Table 2. Diabetes and cardiovascular diseases in Cohort meta-analyses

Study	Design	Population characteristics	Exposure	Outcome(s)	Total follow-up duration
Jin et al., 2018 [49]	Cohort study	96,110 participants of the Kailuan study	Fasting blood glucose	Intracerebral hemorrhagic stroke	9 years
Jin et al., 2017 [50]	Cohort study	68,297 participants without diabetes who were free of MI, Stroke, and cancer	Fasting blood glucose	Myocardial infarction	4 years
Park et al., 2013 [51]	Cohort study	A total of 1,197,384 Korean adults with no specific medical condition.	Fasting blood glucose	ASCVD IHD Stroke	16 years
Zhang et al., 2021 [52]	Cohort study	A total of 16,113 participants with no specific medical condition	Fasting blood glucose	Stroke	5.5 years
Shaye et al, 2012 [53]	Cohort study	Data from 10,913 apparently healthy men and women	Fasting blood glucose	ASCVD	4.3 years
Brutsaert et al, 2016 [54]	Cohort study	Data from 5,201 healthy individuals were recruited in 1989-1990	Fasting blood glucose	ASCVD	11.2 years

Folsom et al, 1997 [55]	Cohort study	15,792 ARIC study pariticipants (ARIC study)	Fasting blood glucose	CHD	4-7 years
Yarnell et al, 1994 [56]	Cohort study	4860 middle aged men from South Wales and Bristol	Fasting blood glucose	IHD	38-61 months

ASCVD, atherosclerotic cardiovascular disease; IHD, ; CHD, ;MI, Myocardial infarction;

4. Association between Diabetes and cardiovascular diseases from Meta analysis of cohort studies

4-1. Analysis of relevance per 10 mg/dl increase using GLIST

4-1-1. Full data analysis

Since the diseases reported in each paper were ASCVD, IHD, and Stroke, we first conducted an overall analysis combining all of them. Through a total of 17 extracted data, we looked at the relationship between fasting blood sugar and overall cardiovascular and cerebrovascular events per 10 mg/dL increase. In the random effect model, OR=1.039 (95%CI=1.028-1.050).

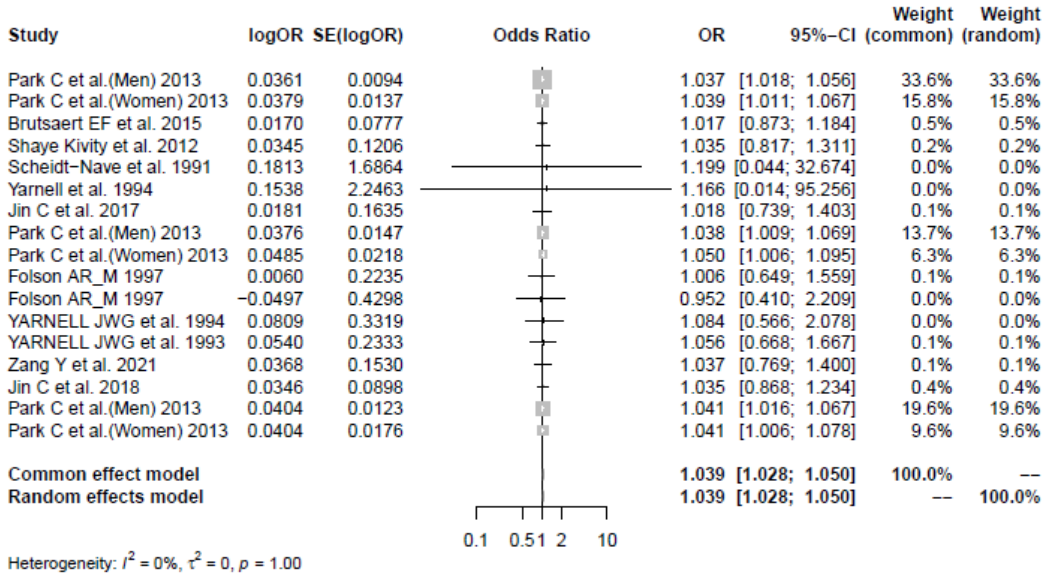

Figure 6. Meta-analysis results for the association between Fasting blood glucose and ASCVD, IHD, Stroke in cohort studies

4-1-2. Meta analysis on ASCVD

As a result of confirming the relationship between fasting blood sugar and IHD per 10 mg/dL increase using 6 data out of a total of 17 extracted data through a random effects model, it was found that the probability of IHD occurring increased by 4% when fasting blood sugar increased by 10 mg (OR =1.037, 95%CI=1.022-1.053).

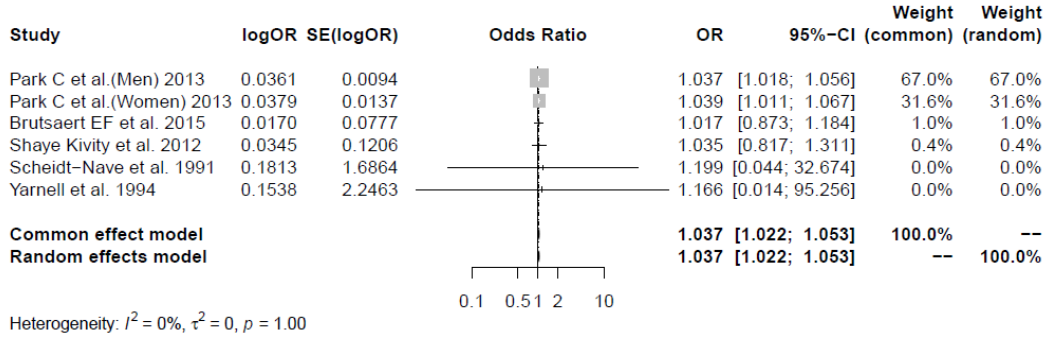

Figure 7. Meta-analysis results for the association between Fasting blood glucose and ASCVD in cohort studies

4-1-3. Meta analysis on IHD

As a result of confirming the relationship between fasting blood sugar and IHD per 10 mg/dL increase using 6 data out of a total of 17 extracted data through a random effects model, it was found that the probability of IHD occurring increased by 4% when fasting blood sugar increased by 10 mg (OR =1.042, 95%CI=1.017-1.067).

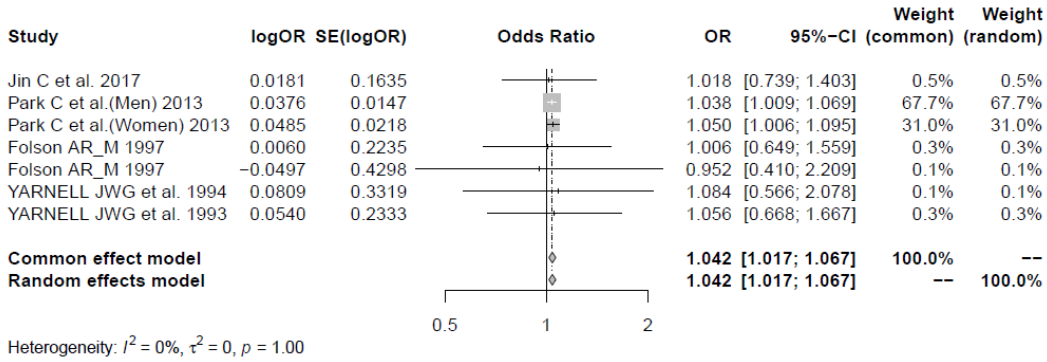

Figure 8. Meta-analysis results for the association between Fasting blood glucose and IHD in cohort studies

4-1-4. Meta analysis on stroke

As a result of confirming the relationship between fasting blood sugar and stroke per 10 mg/dL increase using 4 data out of a total of 17 extracted data through a random effects model, it was found that the probability of stroke occurring increased by 4% when fasting blood sugar increased by 10 mg (OR=1.041, 95%CI=1.021-1.062).

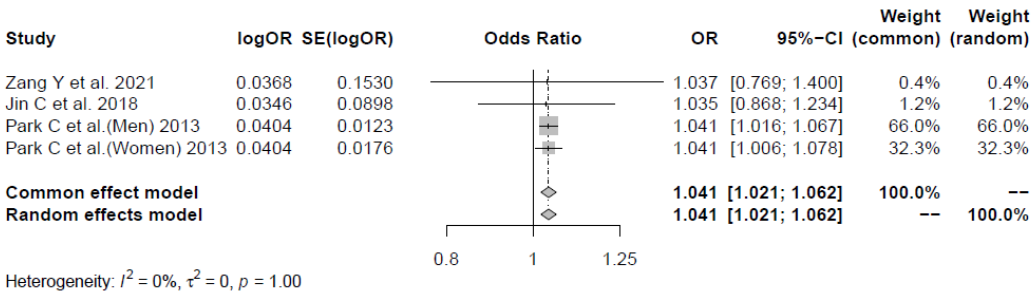

Figure 9. Meta-analysis results for the association between Fasting blood glucose and Stroke in cohort studies

PART 2. Observational Study(Cohort)

1. General characteristics

A total of 153,977 Korean participants with genetic information were used in the analysis. The average follow-up period was 13 years and the number of ASCVD cases was 11,588. Among those who developed ASCVD, the proportion of women was 38.9%, with a higher incidence rate among men, and the average age of onset was 53.8 years. The fasting serum glucose level was about 11 points higher than the control group, and body weight, waist circumference, systolic blood pressure, LDL-cholesterol, and triglyceride were all higher than the control group. Compared to the general population, the income of the ASCVD group was lower, the smoking rate was higher, and the physical activity and drinking rates were lower. Baseline characteristics are shown in Table 1.

Table 3. Baseline characteristics of participants in the KCPS-II study of 153,971

Variable	ASCVD	Control	p-value
	11,588	142,383	
Female (%)	34.0	40.0	<0.001
Age and socioeconomic factors			
Age (years)	52.9 ± 11.4	40.8 ± 10.0	<0.001
School education year	12.7 ± 3.8	14.4 ± 3.0	<0.001
Monthly income, x10,000 won	350 ± 213	434 ± 202	<0.001
Lifestyle factors			
Ever smoking status (%)	55.9	50.4	<0.001
Alcohol drinking status (%)	73.0	84.4	<0.001
Physical activity status (%)	36.0	41.8	<0.001
Anthropometry and BP			
Fasting serum glucose (mg/dl)	101 ± 30.1	90.2 ± 17.5	<0.001
Height (cm)	164 ± 8.6	165 ± 8.3	<0.001
Weight (kg)	67.2 ± 11.1	63.6 ± 12.0	<0.001
BMI (kg/m^2)	24.8 ± 3.10	23.2 ± 3.21	<0.001
Waist circumference (cm)	85.3 ± 8.96	78.9 ± 9.75	<0.001
Systolic BP (mmHg)	126 ± 15.8	116 ± 14.4	<0.001
Lipid profiles			
LDL cholesterol (mg/dl)	114 ± 34.3	111 ± 31.0	<0.001
HDL cholesterol (mg/dl)	50.1± 11.4	53.6 ± 11.3	<0.001
Triglycerides (mg/dl)	155 ± 104	125 ± 85.2	<0.001
Self-reported disease			
HTN (%)	53.9	15.0	<0.001
DM (%)	17.4	4.4	<0.001

BMI, body mass index; BP, blood pressure; LDL, low-density lipoprotein; HDL, high-density lipoprotein; HTN, hypertension and; DM, diabetes mellitus.
Analyzed using independent t-test for categorical variables. Analyzed using the chi-square test for continuous variables.
Data are expressed as mean ± SD unless otherwise indicated.

2. Cox Proportional-Hazards Model

Table 3 shows the risk ratio of fasting serum glucose level for cardiovascular disease after adjusting for sex, age, HDL-cholesterol, LDL-cholesterol, systolic blood pressure, drinking status, and smoking status. Overall, there was an increased risk of developing all cardiovascular diseases except hemorrhagic stroke. It was estimated that for each unit increase in fasting serum glucose level, the risk of ASCVD and stroke increases by 4% (HR_{ASCVD}=1.049, 95% CI_{ASCVD}=1.039-1.059/HR_{TOSTR}=1.042, 95%CI_{TOSTR}=1.027-1.056). The risk of thrombotic stroke increased by 6% (HR_{TRSTR}=1.064, 95% CI_{TRSTR}=1.046-1.083), ischemic heart disease increased by 5% (HR_{IHD}=1.054, 95% CI_{IHD}=1.043-1.065), and myocardial infarction increased by 7% (HR_{MI}=1.078, 95% CI_{MI}=1.054-1.102).

Table 4. Association of FSG with ASCVD using Cox proportional hazard analysis

Exposure variable	Exposure(n) Total 153,274	Cox proportional hazard Analysis*	
		HR (95% CI)	p-value
FSG (per 10mg/dL)	ASCVD(6,609)	1.049(1.039-1.059)	<.0001
	TOSTR(3,255)	1.042(1.027-1.056)	<.0001
	TRSTR(1,451)	1.064(1.046-1.083)	<.0001
	HRSTR(633)	1.026(0.990-1.062)	0.1610
	IHD(4,478)	1.054(1.043-1.065)	<.0001
	MI(775)	1.078(1.054-1.102)	<.0001

HR, Hazard ratio; CI, confidence interval; FSG, fasting serum glucose; ASCVD, atherosclerotic cardiovascular disease; TOSTR, total stroke; TRSTR, thrombotic stroke; HRSTR, hemorrhagic stroke; IHD, ischemic heart disease; MI, myocardial infarction.
*Adjusted for Age, Sex, HDL-Cholesterol, LDL-Cholesterol, Systolic blood pressure, Alcohol status, Smoking status

PART 3. Mendelian randomization

1. Standard (one-sample) MR

Standard Mendelian randomization to investigate whether fasting serum glucose level has a causal effect on ASCVD using WGRS of SNPs with high correlation with fasting serum glucose level discovered through GWAS analysis using KCPS-II data. was performed (Table 3). After adjusting for age and gender, the F statistic for the association between WGRS and fasting blood glucose levels was very high at 3176.47, indicating that WGRS is a valid tool for fasting blood glucose levels. Fasting serum glucose level was found to have an effect on the occurrence of ASCVD (HR_{1SMR}=1.134, 95%CI_{1SMR}=1.038-1.238), similar to observational study results, and had an effect on other cardiovascular diseases in addition to ASCVD. However, unlike the observational study results, the MR study showed that myocardial infarction was not affected by fasting serum glucose.

As a result of additional analysis using FSG-related genetic variants discovered by KoGES, another consortium for Koreans, there was a statistically significant association between ASCVD and HRSTR (OR_{ASCVD} =1.015, 95%CI_{ASCVD} =1.002-1.028, OR_{HRSTR} =1.056, 95 %CI_{HRSTR} =1.008-

1.106) (Appendix 11). Additional analysis was performed using FSG-related genetic variants discovered by the Japanese consortium BBJ, and as a result of KoGES, there was a statistically significant association between ASCVD and HRSTR (OR_{ASCVD} =1.026, 95%CI_{ASCVD} =1.013-1.038; OR_{HRSTR} =1.048, 95%CI_{HRSTR} =1.002-1.096). In the BBJ results, TRSTR showed a significant association with Borderline (OR_{TRSTR} =1.032, p-$value_{TRSTR}$ =0.054) (Appendix 12).

In the analysis using only commonly discovered variants in KCPS-II, KoGES, and BBJ, only ASCVD showed a statistically significant association (OR_{ASCVD} =1.013, 95%CI_{ASCVD} =1.001-1.025) (Table 5).

Table 5. Association of fasting serum glucose with ASCVD using Standard Mendelian randomization

Exposure variable	Exposure(n) Total 153,274	Mendelian Randomization Analysis*		
		F-statistic of WGRS	HR (95% CI)	p-value
FSG (per 10mg/dL)	ASCVD(6609)	3176.47	1.134(1.038-1.238)	0.0055
	TOSTR(3255)		1.149(1.013-1.304)	0.0303
	TRSTR(1451)		1.230(1.017-1.486)	0.0325
	HRSTR(633)		1.199(0.900-1.596)	0.2146
	IHD(4478)		1.179(1.059-1.313)	0.0027
	MI(775)		0.997(0.99-1.003)	0.3662

HR, Hazard ratio; CI, confidence interval; FSG, fasting serum glucose; WGRS, Weighted genetic risk score; ASCVD, atherosclerotic cardiovascular disease; TOSTR, total stroke; TRSTR, thrombotic stroke; HRSTR, hemorrhagic stroke; IHD, ischemic heart disease; MI, myocardial infarction.
*Adjusted for age, sex

Table 6. Causality between fasting serum glucose and ASCVD analyzed with genetic variants commonly discovered in three cohorts(KCPS-II, KoGES, BBJ)

Outcome	F-statistic of WPRS(EAS)	OR (95% CI)	p-value
ASCVD	1123 (n of SNP=15)	1.013 [1.001, 1.025]	0.029
AMI		1.014 [0.961, 1.070]	0.604
IHD		1.000 [0.979, 1.023]	0.970
TOSTR		1.005 [0.985, 1.026]	0.607
TRSTR		1.016 [0.986, 1.048]	0.292
HRSTR		1.032 [0.989, 1.077]	0.147

EAS, East Asian population; OR, Odds ratio; CI, confidence interval; FSG, fasting serum glucose; WGRS, Weighted genetic risk score; ASCVD, atherosclerotic cardiovascular disease; TOSTR, total stroke; TRSTR, thrombotic stroke; HRSTR, hemorrhagic stroke; IHD, ischemic heart disease; MI, myocardial infarction.
*Adjusted for age, sex

2. Two sample MR

Fasting serum glucose genetic variants from KoGES were used as Exposure, and cardiovascular disease prevalence data from KCPS-II were used as Outcome. When evaluating the association with cardiovascular disease, 37 fasting glucose-related genetic variants were selected (Appendix 10A). Assuming no heterogeneity in instrumental variables, fasting blood glucose was significantly associated with ASCVD, IHD, and AMI. Specifically, the OR per 10 mg/dL increase in fasting blood glucose in ASCVD was 1.013 and was statistically significant (95% CI=1.006-1.019, p-value=<0.001).

Cochran's Q test and MR-Egger intercept test will be additionally conducted to analyze whether there is heterogeneity in the instrumental variables [36].

For replication analysis, fasting serum glucose genetic variants from KoGES and KCPS-II were used as exposure values, and MEGASTROKE consortium data were used as cardiovascular disease outcome data. A total of 5 strokes including trans-ethnics' any stroke, any ischemic stroke, large artery stroke, cardioembolic stroke, and small vessel stroke were analyzed, and there were no statistically significant results.

Table 7. Association of fasting serum glucose with ASCVD using Two-sample Mendelian randomization (KoGES, KCPS2)

Outcome	Inverse variance weighted		Weighted median		MR-Egger		MR-PRESSO[1]		*P* for pleiotropy[2]	*P* for global[3]
	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*		
Atherosclerotic cardiovascular disease	1.11(1.04-1.18)	5.45E-05	1.07 (0.98-1.16)	1.26E-01	0.95(0.79-1.16)	6.66E-01	_	_	1.11E-01	0.292
Total stroke	1.05(0.93-1.18)	6.15E-02	1.05(0.91-1.24)	1.53E-01	0.76(0.52-1.09)	1.53E-01	_	_	7.68E-02	0.225
Thrombotic stroke	1.08(0.89-1.31)	4.11E-01	1.02(0.81-1.31)	8.23E-01	0.66(0.37-1.17)	1.68E-01	_	_	8.68E-02	0.102
Hemorrhagic stroke	1.18(0.94-1.48)	1.34E-01	1.15(0.84-1.57)	3.65E-01	0.86(0.42-1.75)	6.91E-01	_	_		0.612
Ischemic heart disease	1.15(1.05-1.26)	1.56E-03	1.12(0.99-1.28)	5.92E-02	1.23(0.92-1.65)	1.62E-01	_	_	6.43E-01	0.226
Myocardial infarction	1.37(1.03-1.83)	3.31E-02	1.28(0.95-1.74)	4.72E-02	1.96(0.79-4.85)	1.52E-01	1.43(1.21-1.66)	3.91E-03	4.19E-01	<0.001

1 The outlier-adjusted causal estimates were presented for associations where both the global and distortion tests provided evidence of horizontal pleiotropy. 2 From MR Egger intercept test, detection of directional pleiotropy. 3 From MR-PRESSO global test, detection of horizontal pleiotropy. CI: confidence interval; OR: odds ratio.

3. Multivariable MR

In this study, multivariable Mendelian randomization (MVMR) used KOGES data for fasting blood sugar, and KCPS-II data for ASCVD, IHD, and stroke data. The F value was presented to evaluate the validity of instrumental variables in the MVMR model. In addition, the crude MR results were compared by presenting the IVW method and the MVMR results side by side. Variables included in MVMR included fasting blood sugar level of 10 mg/dL, systolic blood pressure, and LDL cholesterol.

3-1. MVMR analysis results for ASCVD

The conditional F-statistics for instrument strength values of FSG, SBP, and LDL used in the MVMR model were 13.69, 7.41, and 38.50. Q-Statistic for instrument validity was 141.67 on 121 DF (p=0.096). FSG showed a significantly positive causal relationship with both crude MR and MVMR. The size of the relationship increased as both SBP and LDL showed wide confidence intervals in MVMR.

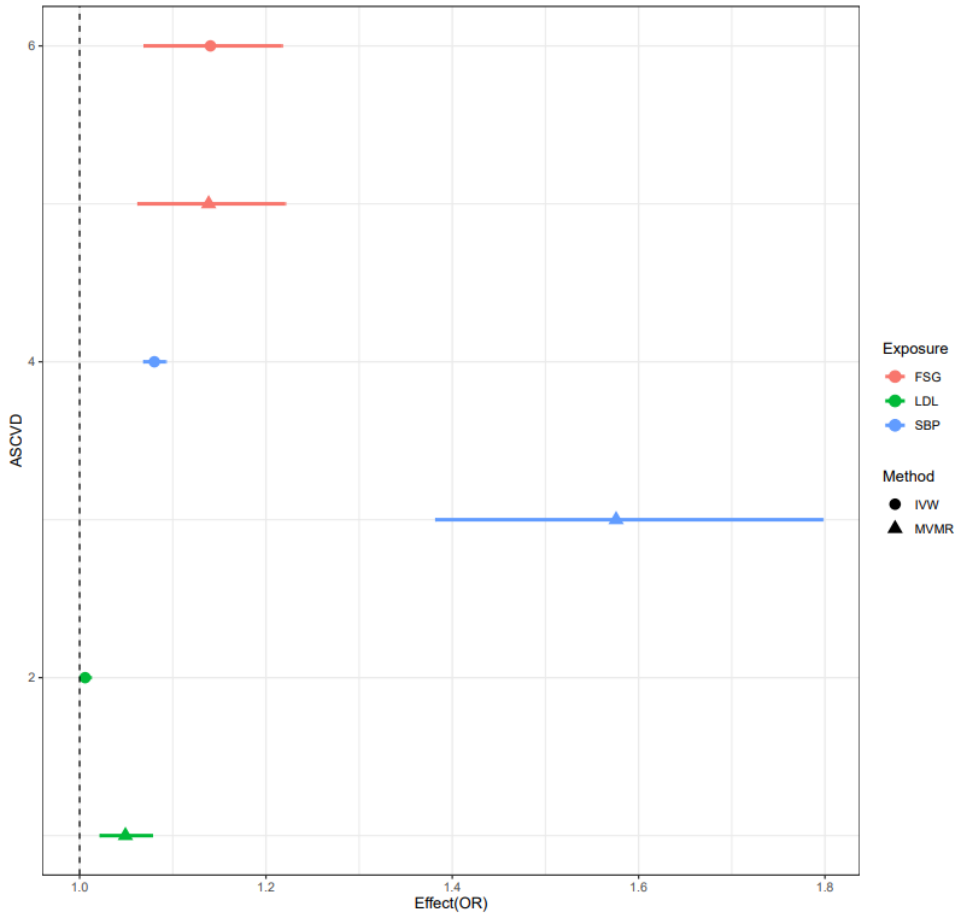

Figure 10. MVMR analysis results for ASCVD

3-2. MVMR analysis results for IHD

The conditional F-statistics for instrument strength values of FSG, SBP, and LDL used in the MVMR model were 13.69, 7.41, and 38.50. Q-Statistic for instrument validity was 142.30 on 121 DF (p=0.090). FSG showed a significantly positive causal relationship with both crude MR and MVMR. The size of the relationship increased as both SBP and LDL showed wide confidence intervals in MVMR.

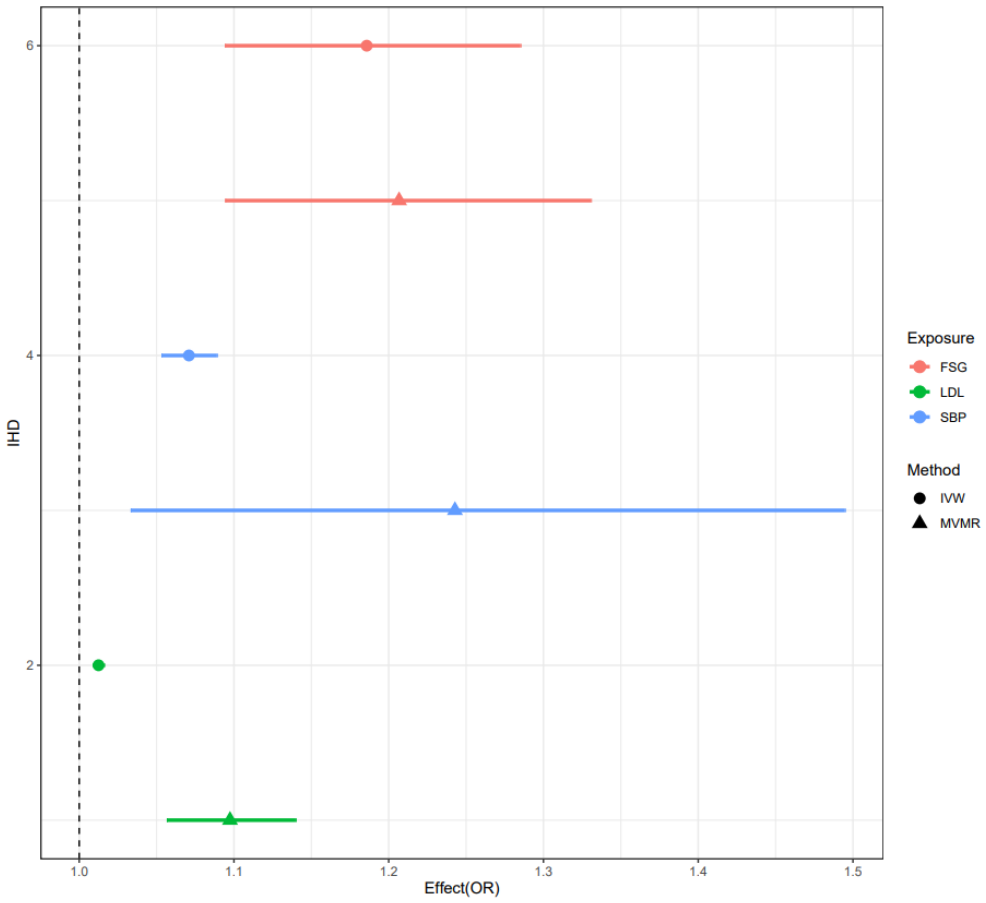

Figure 11. MVMR analysis results for IHD

3-3. MVMR analysis results for stroke

The conditional F-statistics for instrument strength values of FSG, SBP, and LDL used in the MVMR model were 13.69, 7.41, and 38.50. Q-Statistic for instrument validity was 134.33 on 121 DF (p=0.1922). FSG showed no significant causal relationship with both crude MR and MVMR. Both SBP and LDL showed wide confidence intervals for MVMR and were not significant.

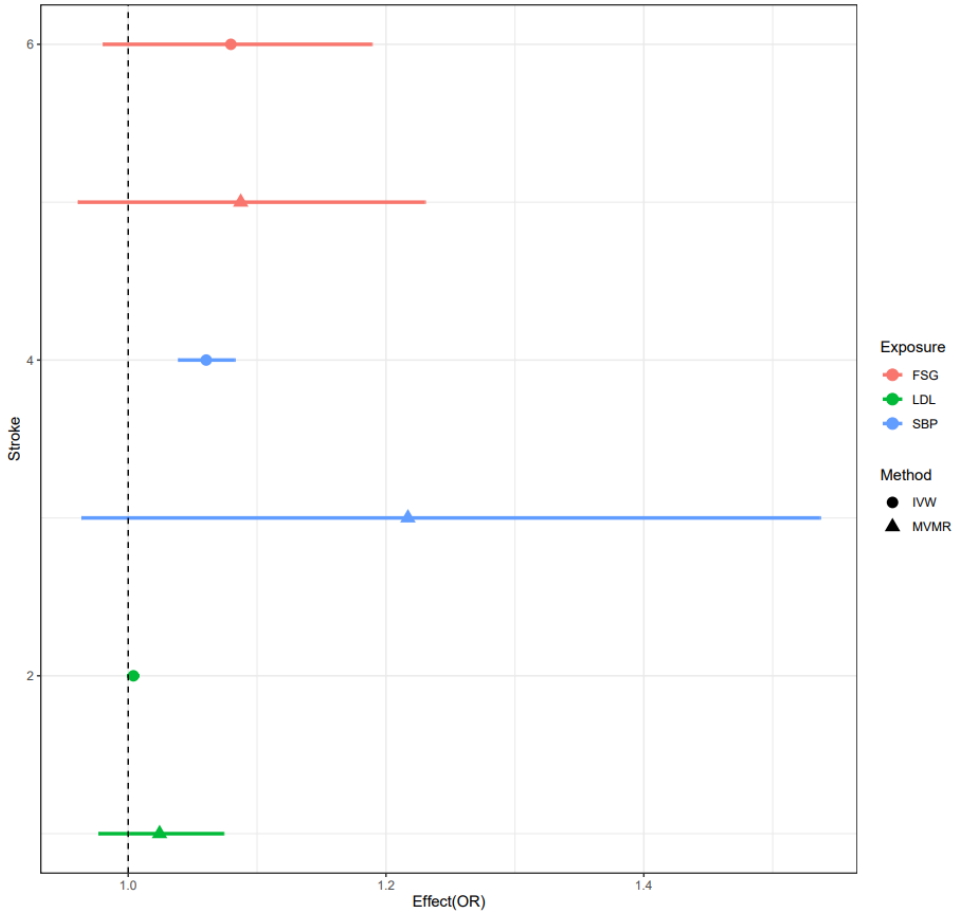

Figure 12. MVMR analysis results for Stroke

PART 4. Triangulation

When looking at the effect of fasting serum glucose on the occurrence of ASCVD using three research methods, the effect size was smaller in the order of meta-analysis using cohort studies, cohort study, MR, but all showed one direction and were statistically significant (Figure 13).

In the results of meta-analysis and cohort analysis, overall, the risk of cardiovascular disease increased by approximately 4% for every 10 mg/dL increase. However, in MR analysis, the risk of cardiovascular disease increased by more than 10% per 10 mg/dL increase in fasting blood sugar. This is considered to be a reduced result from an observational study due to confounding variables or reverse causation.

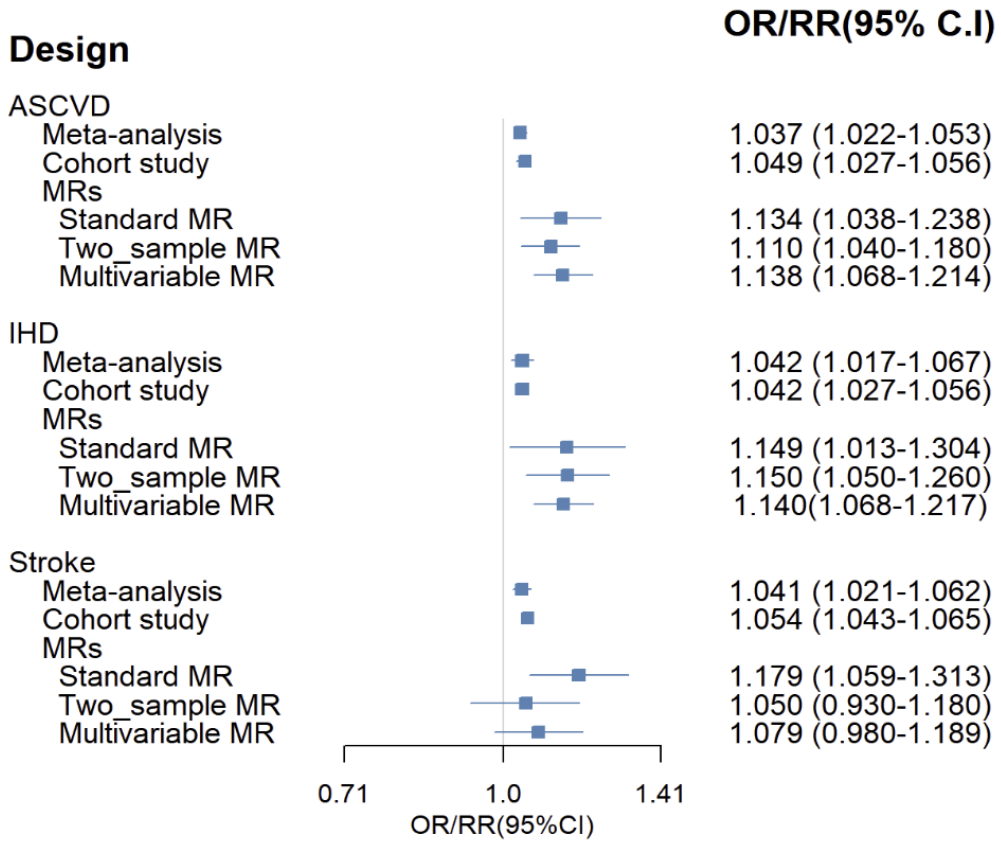

Figure 13. Association between fasting blood glucose measured by various methods and ASCVD for triangulation

Ⅳ. DISCUSSION

4.1 Key findings

Fasting blood glucose levels are known to influence the development of cardiovascular diseases, but the causality or direction of the association has been uncertain. This study used a triangular approach to demonstrate that FSG levels are causally related to ASCVD in the Korean population. First, as a result of a meta-analysis of clinical trial studies on lifestyle improvement interventions for diabetes, it was confirmed that the probability of developing heart disease or dying from heart disease was 17% lower in the intervention group. Second, as a result of meta-analysis of the cohort study, the risk of ASCVD increased by 3.7% for every 10 mg/dL increase in FSG. Third, in a cohort study, Cox regression analysis was performed between fasting serum glucose and cardiovascular events, and the results showed that fasting serum glucose increased the risk of ASCVD and stroke by 4%, and also had a statistically significant association with thrombotic stroke, ischemic heart disease, and myocardial infarction. there was. Fourth, as a result of the standard MR study, when instrumental variables discovered from KCPS-II were used, genetically determined

fasting serum glucose had a causal relationship with ASCVD, stroke, thrombotic stroke, and ischemic heart disease. Fifth, as a result of a two-sample MR study, genetic variants related to fasting serum glucose discovered in KoGES were used as instrumental variables to confirm the correlation with cardiovascular disease prevalence data of KCPS2, and the analysis results showed that ASCVD, AMI, and IHD were statistically significant with fasting serum glucose. Lastly, in MVMR controlling for SBP and LDL, ASCVD and IHD increased by 13.8% and IHD by 14.0%, respectively, for every 10 mg/dL increase in FSG. However, the risk of stroke did not increase for each 10 mg/dL increase in FSG.

4.2 Previous studies

One of the diagnostic criteria for type 2 diabetes is FSG. Clinical data, on the other hand, suggest that not all modest physiological elevations in FSG are associated with type 2 diabetes[57] and that even after 15 years of exposure to increased FSG concentrations, not all individuals progress to type 2 diabetes[58]. Although type 2 diabetes has long been recognized as a CAD risk factor, the complex interaction of several metabolic changes adds to the difficulty in determining whether the glycemic component affects CAD risk[59]. Epidemiologic studies in this area are prone to

confounding, and randomized controlled trials for glycemic control have been inconclusive. Genetic analyses that include a GRS as a polygenic instrument in an MR framework can capture a sufficient proportion of variance in an endophenotype and establish causality for that trait. The inclusion of nonoverlapping FSG and type 2 diabetes variants is a key novel feature of the current study. In this study, we looked at one aspect of type 2 diabetes, hyperglycemia, while attempting to rule out other factors associated with a complex disease process involving mechanisms other than hyperglycemia. Our findings support the hypothesis that glucose elevation is a significant contributor to increased ASCVD.

A clinical implication of our findings is that they lend credence to the notion that lowering glucose may confer cardiovascular benefits even among individuals without diabetes. Prospective epidemiological data have consistently shown that even small changes in blood glucose can have an effect on cardiovascular morbidity and mortality in healthy participants[60]. In an extended meta-regression analysis, a linear relationship with no threshold effect was observed between FSG and CAD risk (the relative cardiovascular event risk reduction was 1.33 compared with FSG concentrations of 4.2 vs. 6.1 mmol/L)[2]. In addition, intensive glucose-lowering therapy reduced the risk of myocardial infarction in people with

newly diagnosed type 2 diabetes[61] and in patients with established type 2 diabetes by 15 to 20%[62]. A meta-analysis of large RCTs found intensive glucose-lowering was associated with a significant 9% reduction in CAD events[63].

4.3 Mechanisms of fasting serum glucose and CVD

Our findings are consistent with previous observations that plasma glucose elevations can directly aggravate structural changes in the arterial wall through a variety of mechanisms, including endothelial dysfunction, vascular smooth muscle cell proliferation, and an inflammatory phenotype change in macrophages [64].

Fasting serum glucose levels and cardiovascular disease (CVD) are closely interconnected, and several mechanisms explain their relationship. Fasting serum glucose, often measured as fasting plasma glucose (FPG), is an important indicator of glucose regulation and can have a significant impact on cardiovascular health. Here are some of the mechanisms that link fasting serum glucose and cardiovascular disease:

1. Insulin Resistance: High fasting serum glucose levels are often associated with insulin resistance [65], where the body's cells do not respond

effectively to insulin, a hormone responsible for regulating blood sugar. This condition can lead to higher FSG levels, which in turn can contribute to CVD risk factors, such as hypertension and dyslipidemia [66].

2. Atherosclerosis: Elevated FSG levels are linked to the development and progression of atherosclerosis, a condition in which fatty deposits accumulate in the arteries, leading to reduced blood flow. Chronic hyperglycemia can cause damage to the blood vessel walls, promoting atherosclerosis and increasing the risk of heart disease [67].

3. Inflammation: High blood sugar levels can lead to chronic low-grade inflammation in the body. Inflammation is a key driver of atherosclerosis and can increase the risk of cardiovascular events. Elevated FSG levels may contribute to this inflammatory state[68].

4. Dyslipidemia: Abnormal fasting serum glucose levels can disrupt lipid metabolism, leading to an unfavorable lipid profile, characterized by higher levels of triglycerides and lower levels of high-density lipoprotein (HDL) cholesterol. These lipid abnormalities can increase the risk of CVD [69, 70].

5. Oxidative Stress: High blood sugar levels can increase oxidative stress, which is the imbalance between the production of harmful free radicals and the body's ability to counteract their effects. Oxidative stress can damage blood vessels and contribute to CVD [71, 72].

6. Endothelial Dysfunction: Chronic hyperglycemia can impair the function of the endothelium, the inner lining of blood vessels. Endothelial dysfunction is a key step in the development of atherosclerosis and can lead to hypertension and reduced vascular flexibility, both of which are risk factors for CVD [73].

7. Platelet Aggregation: Elevated blood sugar levels may promote abnormal platelet aggregation, increasing the risk of blood clots and thrombotic events, which can cause heart attacks and strokes [74, 75].

8. Advanced Glycation End Products (AGEs): High glucose levels can lead to the formation of advanced glycation end products, which can damage

proteins and lipids in the blood vessels and contribute to CVD development [76].

9. Autonomic Nervous System Dysfunction: Hyperglycemia can affect the autonomic nervous system, leading to alterations in heart rate variability and blood pressure regulation, which can increase the risk of arrhythmias and other cardiovascular issues [77].

10. Metabolic Syndrome: Elevated fasting serum glucose levels are often part of the metabolic syndrome [78], a cluster of risk factors that increase the likelihood of developing CVD. Metabolic syndrome includes factors like obesity, high blood pressure, and dyslipidemia, all of which are influenced by blood sugar regulation [79].

To mitigate the risk of cardiovascular disease associated with elevated fasting serum glucose levels, individuals are encouraged to manage their blood sugar through lifestyle changes (diet and exercise) [80], medication (when necessary), and regular medical check-ups. Maintaining a healthy

diet, staying physically active, and managing stress can help control blood sugar levels and reduce the risk of CVD.

4.4 Strengths and limitations

The association between fasting serum glucose and ASCVD was confirmed using data from four large-scale East Asian Biobank Consortiums.

Strengths of our study include the reduction of concerns about confounding and reverse causation by applying an MR approach, as genetic variants are fixed at conception and less associated with confounders than directly measured environmental exposures [81]. Even with a genetic instrument comprised of variants derived from different populations, our validation analysis revealed the same causal relationship between FSG and ASCVD. The described results are also consistent with the findings of a recent MR study that excluded the effect of pleiotropy [82].

Our findings should be interpreted with caution because other unmeasured factors could skew our estimate of elevated FSG on ASCVD. In this study, the MR method was used to solve this problem. However, the MR method used in this study, especially MVMR, had a limitation in that the F value of SBP as an instrumental variable was not sufficient.

We also recognize that nongenetic factors may play a larger role in FSG and ASCAD risk. In this study, genetically determined FSG was used to solve this problem. In the future, research considering genetic-environmental factors and interactions will be needed.

MR studies make several assumptions, such as pleiotropy and linkage disequilibrium confounding, population structure, or weak genetic instruments [83].

Our MR variable analysis also assumed a linear relationship between FSG and ASCVD. FSG may have a non-linear relationship with ASCVD, but this is typically seen in individuals with low glucose levels, who represent a minority of the general population [84].

Finally, we cannot confirm that our findings apply to other ethnic groups because our research was limited to Korean populations.

V. CONCLUSION

In conclusion, our findings support the causal relationship between increased FSG and ASCVD risk in the Korean population. In meta-analysis and cohort studies through observational studies, the effect of ASCVD per 10 mg/dl increase in FSG was lower than the effect of ASCVD per 10 mg/dl increase in FSG in MR analysis. It is believed that true causal association is suppressed due to the influence of confounding variables or reverse causation in observational studies.

More experimental studies and larger and longer prospective genetic epidemiologic studies are needed to fully understand the biological and molecular mechanisms of hyperglycemia and its association with the pathogenesis of ASCVD by gene site.

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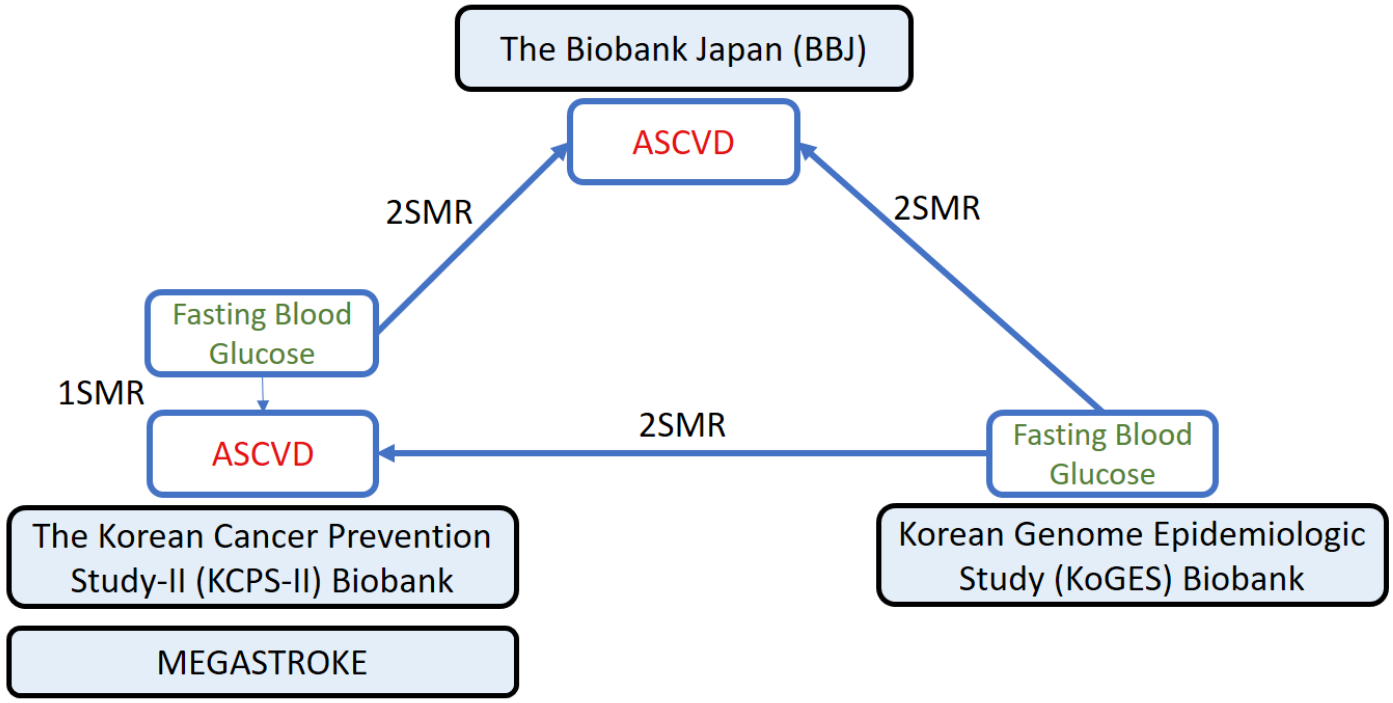
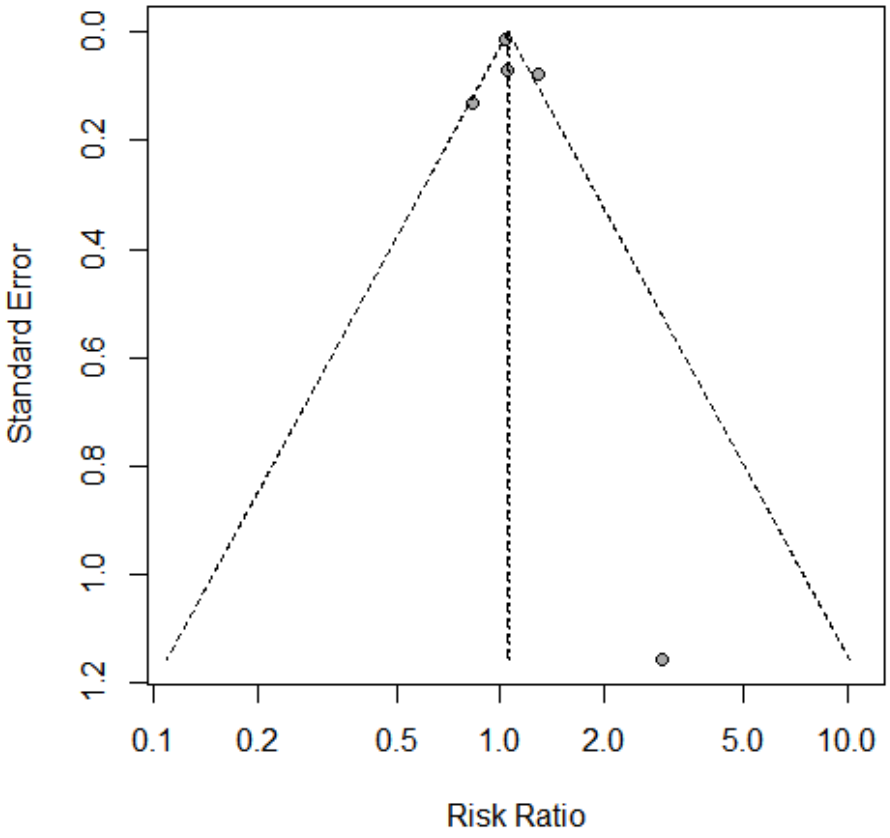
Appendix 1. Research promotion system utilizing 4 biobanks



ASCVD, Atherosclerotic cardiovascular disease; 1SMR, One-sample Mendelian randomization; 2SMR, Two-sample Mendelian randomization



Appendix 2. Funnel plot of Meta analysis(RCTs)

Appendix 3. Mendelian randomization analysis for the effects of fasting serum glucose on ASCVD risk using chromosomal GRS (CHR2,3,4)

Exposure variable	Exposure(n) Total 153274	CHR2 F=544.32		CHR3 F=163.77		CHR4 F=105.15	
		HR	p-value	HR	p-value	HR	p-value
FSG (per 10mg/dL)	ASCVD(6609)	1.305(1.057-1.611)	0.0135	0.779(0.53-1.143)	0.2020	1.008(0.623-1.631)	0.9752
	TOSTR(3255)	1.172(0.867-1.583)	0.3026	0.972(0.564-1.677)	0.9190	1.213(0.610-2.412)	0.5816
	TRSTR(1451)	1.344(0.857-2.108)	0.1978	0.824(0.364-1.864)	0.6418	1.118(0.400-3.128)	0.8313
	HRSTR(633)	1.232(0.623-2.437)	0.5487	0.504(0.145-1.753)	0.2815	1.354(0.285-6.451)	0.7033
	IHD(4478)	1.588(1.299-2.051)	0.0004	0.815(0.511-1.298)	0.3887	1.206(0.671-2.167)	0.5320
	MI(775)	1.535(0.830-2.841)	0.1723	2.508(0.828-7.597)	0.1041	4.002(0.952-16.816)	0.0583

CHR, Chromosome; HR, Hazard ratio; CI, confidence interval; FSG, fasting serum glucose; WGRS, Weighted genetic risk score; ASCVD, atherosclerotic cardiovascular disease; TOSTR, total stroke; TRSTR, thrombotic stroke; HRSTR, hemorrhagic stroke; IHD, ischemic heart disease; MI, myocardial infarction.
*Adjusted for age, sex

Appendix 4. Mendelian randomization analysis for the effects of fasting serum glucose on ASCVD risk using chromosomal GRS (CHR5,6,7)

Exposure variable	Exposure(n) Total 153274	CHR5 F=23.58		CHR6 F=363.56		CHR7 F=649.88	
		HR	p-value	HR	p-value	HR	p-value
FSG	ASCVD(6609)	0.688(0.247-1.918)	0.4745	0.952(0.735-1.233)	0.7099	1.114(0.919-1.350)	0.2721
	TOSTR(3255)	0.475(0.109-2.069)	0.3214	1.020(0.706-1.473)	0.9158	1.049(0.797-1.380)	0.7338
	TRSTR(1451)	0.636(0.072-5.628)	0.6844	1.151(0.664-1.996)	0.6163	1.031(0.684-1.556)	0.8831
	HRSTR(633)	0.144(0.005-4.570)	0.2719	0.996(0.433-2.293)	0.996	0.636(0.341-1.186)	0.1549
	IHD(4478)	0.886(0.258-3.044)	0.8475	1.040(0.760-1.423)	0.8063	1.170(0.926-1.477)	0.1884
	MI(775)	0.354(0.017-7.451)	0.5039	1.251(0.589-2.657)	0.5603	1.223(0.698-2.146)	0.4818

CHR, Chromosome; HR, Hazard ratio; CI, confidence interval; FSG, fasting serum glucose; WGRS, Weighted genetic risk score; ASCVD, atherosclerotic cardiovascular disease; TOSTR, total stroke; TRSTR, thrombotic stroke; HRSTR, hemorrhagic stroke; IHD, ischemic heart disease; MI, myocardial infarction.
*Adjusted for age, sex

Appendix 5. Mendelian randomization analysis for the effects of fasting serum glucose on ASCVD risk using chromosomal GRS (CHR8,9,10)

Exposure variable	Exposure(n) Total 153274	CHR8 F=209.32		CHR9 F=454.68		CHR10 F=229.41	
		HR	p-value	HR	p-value	HR	p-value
FSG	ASCVD(6609)	1.098(0.782-1.541)	0.5893	1.004(0.797-1.266)	0.9708	1.208(0.872-1.674)	0.2554
	TOSTR(3255)	1.016(0.626-1.648)	0.9502	0.854(0.614-1.188)	0.3499	1.607(1.018-2.535)	0.0417
	TRSTR(1451)	1.656(0.801-3.425)	0.1735	0.586(0.358-0.961)	0.0341	1.663(0.837-3.303)	0.1463
	HRSTR(633)	0.954(0.318-2.857)	0.9325	2.169(1.023-4.595)	0.0433	1.409(0.499-3.981)	0.5176
	IHD(4478)	0.975(0.646-1.472)	0.9036	1.037(0.783-1.374)	0.7987	1.242(0.836-1.845)	0.2836
	MI(775)	0.952(0.353-2.562)	0.9220	1.855(0.942-3.650)	0.0737	3.703(1.525-8.991)	0.0038

CHR, Chromosome; HR, Hazard ratio; CI, confidence interval; FSG, fasting serum glucose; WGRS, Weighted genetic risk score; ASCVD, atherosclerotic cardiovascular disease; TOSTR, total stroke; TRSTR, thrombotic stroke; HRSTR, hemorrhagic stroke; IHD, ischemic heart disease; MI, myocardial infarction.
*Adjusted for age, sex

Appendix 6. Mendelian randomization analysis for the effects of fasting serum glucose on ASCVD risk using chromosomal GRS (CHR11,12,13)

Exposure variable	Exposure(n) Total 153274	CHR11 F=759.88		CHR12 F=268.61		CHR13 F=649.88	
		HR	p-value	HR	p-value	HR	p-value
FSG	ASCVD(6609)	1.081(0.904-1.293)	0.3931	1.260(0.933-1.701)	0.1314	1.196(0.696-2.055)	0.5161
	TOSTR(3255)	0.928(0.729-1.198)	0.5686	1.539(1.000-2.369)	0.0501	1.127(0.521-2.440)	0.7611
	TRSTR(1451)	1.100(0.751-1.613)	0.6246	1.783(0.934-3.405)	0.0797	1.232(0.388-3.913)	0.7231
	HRSTR(633)	0.910(0.510-1.621)	0.7481	2.013(0.746-5.430)	0.1670	1.101(0.191-6.361)	0.9140
	IHD(4478)	1.305(1.050-1.621)	0.0162	1.068(0.744-1.532)	0.7225	1.508(0.782-2.908)	0.2198
	MI(775)	1.377(0.817-2.319)	0.2298	0.129(0.059-0.285)	<.0001	1.479(0.304-7.206)	0.6277

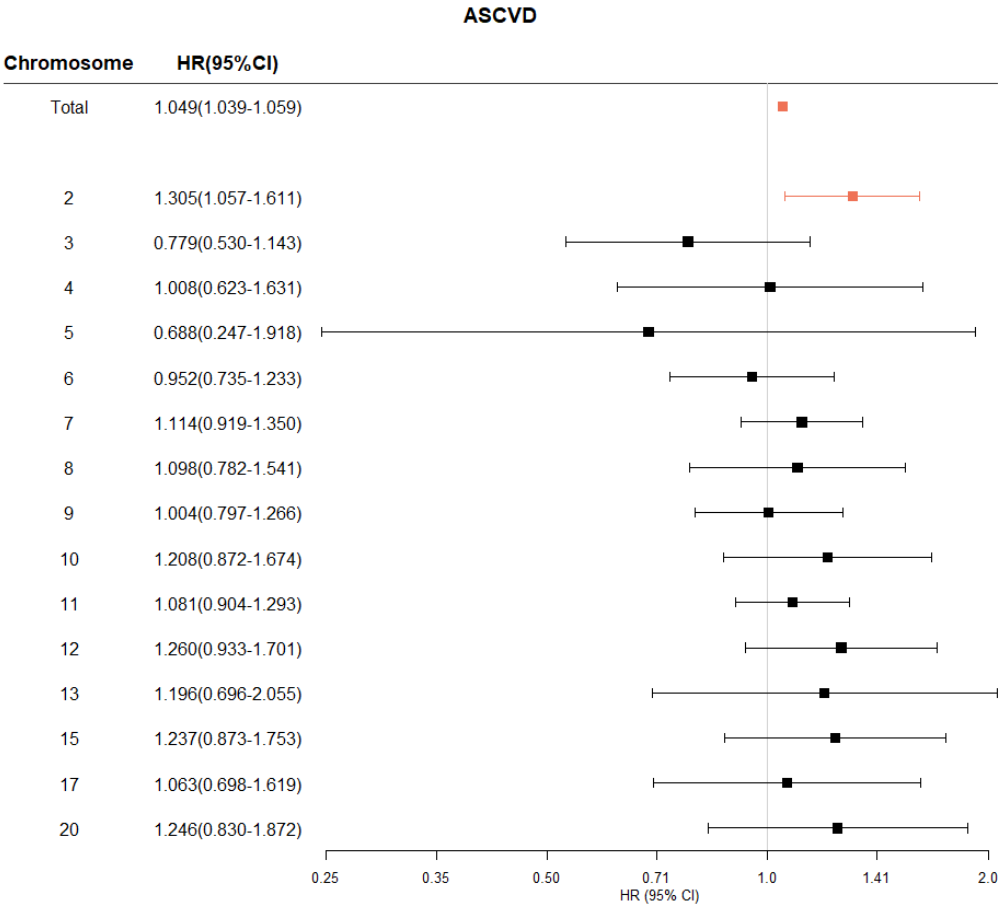
CHR, Chromosome; HR, Hazard ratio; CI, confidence interval; FSG, fasting serum glucose; WGRS, Weighted genetic risk score; ASCVD, atherosclerotic cardiovascular disease; TOSTR, total stroke; TRSTR, thrombotic stroke; HRSTR, hemorrhagic stroke; IHD, ischemic heart disease; MI, myocardial infarction.
*Adjusted for age, sex

Appendix 7. Mendelian randomization analysis for the effects of fasting serum glucose on ASCVD risk using chromosomal GRS (CHR15,17,20)

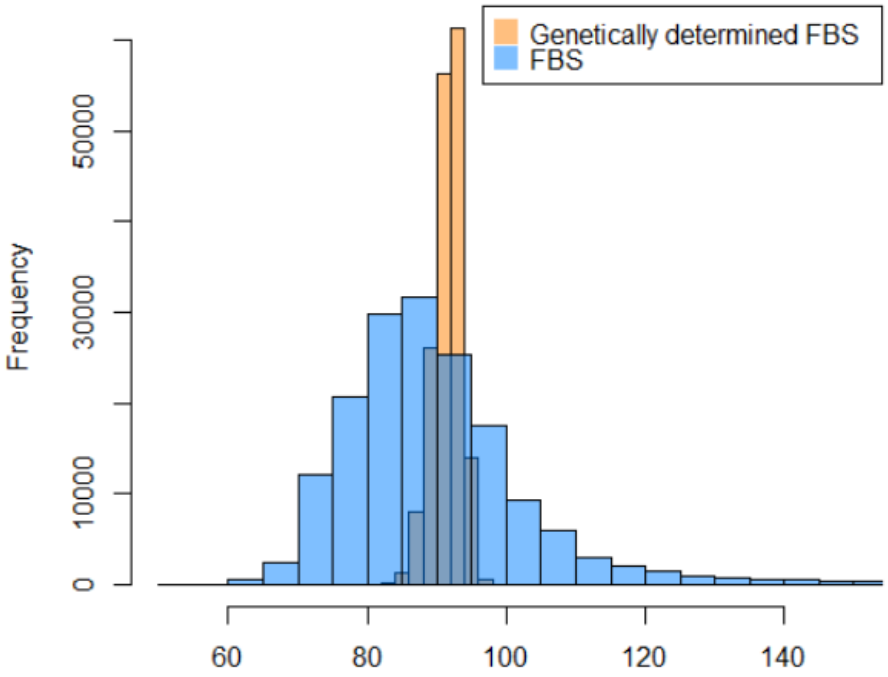
Exposure variable	Exposure(n) Total 153274	CHR15 F=197.78		CHR17 F=135.79		CHR20 F=152.57	
		HR	p-value	HR	p-value	HR	p-value
FSG	ASCVD(6609)	1.237(0.873-1.753)	0.2313	1.063(0.698-1.619)	0.7761	1.246(0.830-1.872)	0.2886
	TOSTR(3255)	1.256(0.764-2.067)	0.3689	1.128(0.620-2.053)	0.6921	1.181(0.663-2.102)	0.5727
	TRSTR(1451)	0.890(0.422-1.873)	0.7583	1.329(0.544-3.247)	0.5325	1.630(0.675-3.936)	0.2774
	HRSTR(633)	1.904(0.615-5.898)	0.2643	0.776(0.198-3.037)	0.7154	1.311(0.353-4.876)	0.6859
	IHD(4478)	1.119(0.733-1.709)	0.6034	1.126(0.676-1.877)	0.6482	1.365(0.831-2.241)	0.2189
	MI(775)	1.314(0.474-3.647)	0.5996	0.833(0.242-2.860)	0.7710	1.598(0.482-5.294)	0.4433

CHR, Chromosome; HR, Hazard ratio; CI, confidence interval; FSG, fasting serum glucose; WGRS, Weighted genetic risk score; ASCVD, atherosclerotic cardiovascular disease; TOSTR, total stroke; TRSTR, thrombotic stroke; HRSTR, hemorrhagic stroke; IHD, ischemic heart disease; MI, myocardial infarction.
*Adjusted for age, sex

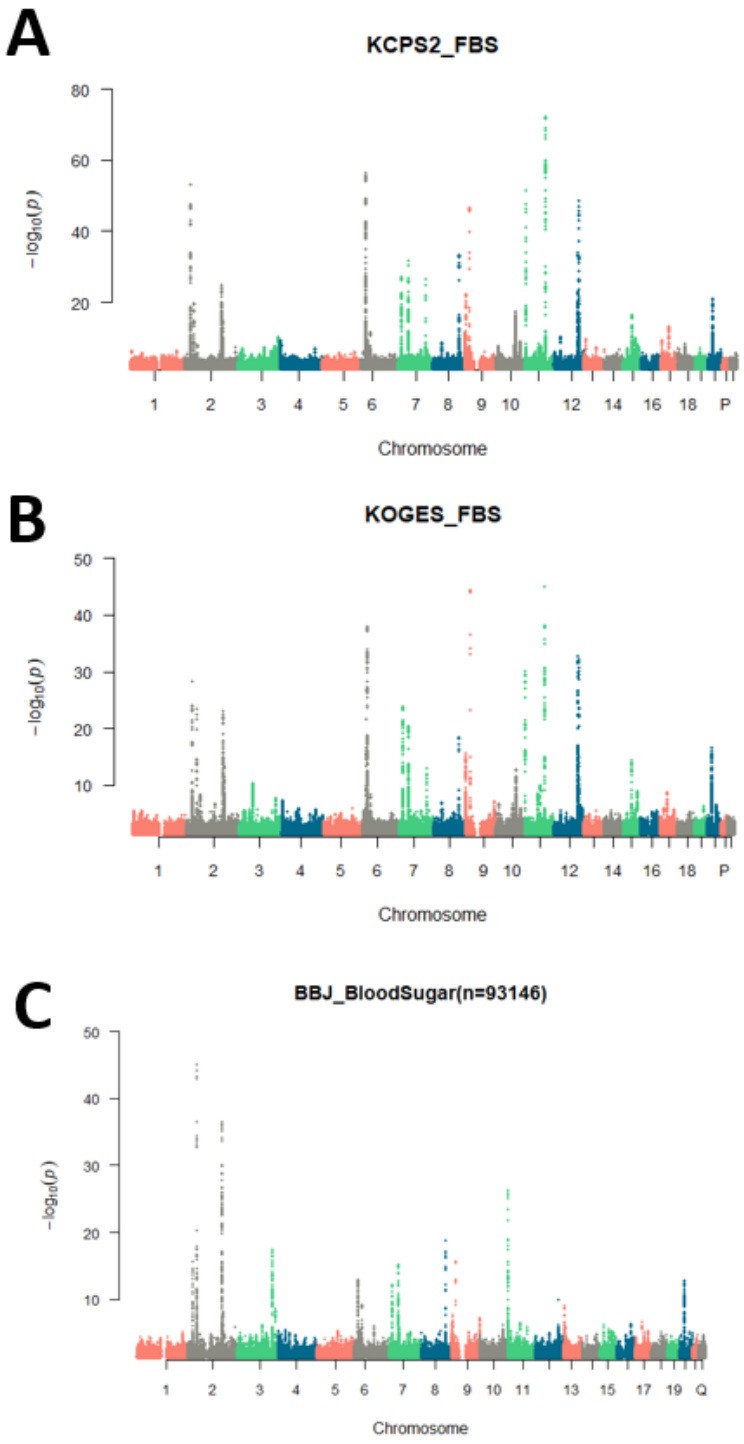
Appendix 8. Forest plot on ASCVD risk affected by fasting serum glucose by chromosome



Appendix 9. Genetically determined fasting serum glucose



Appendix 10. Manhattan plot of Fasting Blood Glucose Analyzed in Three Cohorts (East Asians)



Appendix 11. Causality between fasting serum glucose and ASCVD analyzed using genetic variants discovered in KoGES

Outcome	F-statistic of WGRS(KoGES)	OR (95% CI)	p-value
ASCVD	957.6 (n of SNP=30)	1.015 [1.002, 1.028]	0.021
AMI		1.017 [0.960, 1.078]	0.559
IHD		1.003 [0.979, 1.027]	0.810
TOSTR		1.012 [0.990, 1.035]	0.290
TRSTR		1.007 [0.975, 1.041]	0.661
HRSTR		1.056 [1.008, 1.106]	0.021

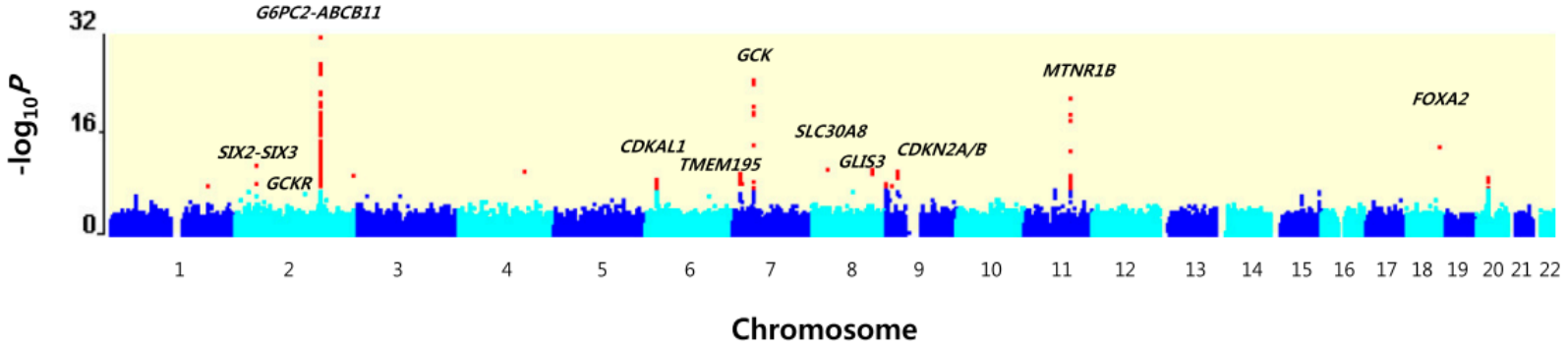
KoGES, The Korean Genome and Epidemiology Study; OR, Odds ratio; CI, confidence interval; FSG, fasting serum glucose; WGRS, Weighted genetic risk score;ASCVD, atherosclerotic cardiovascular disease; TOSTR, total stroke; TRSTR, thrombotic stroke; HRSTR, hemorrhagic stroke; IHD, ischemic heart disease; MI, myocardial infarction.
*Adjusted for age, sex

Appendix 12. Causality between fasting serum glucose and ASCVD analyzed using genetic variants discovered in BBJ

Outcome	F-statistic of WGRS(BBJ)	OR (95% CI)	p-value
ASCVD	1017 (n of SNP=20)	1.026 [1.013, 1.038]	<0.05
AMI		1.027 [0.971, 1.087]	0.348
IHD		1.022 [0.999, 1.046]	0.066
TOSTR		1.019 [0.997, 1.042]	0.083
TRSTR		1.032 [0.999, 1.065]	0.054
HRSTR		1.048 [1.002, 1.096]	0.040

BBJ, Biobank of Japan; OR, Odds ratio; CI, confidence interval; FSG, fasting serum glucose; WGRS, Weighted genetic risk score; ASCVD, atherosclerotic cardiovascular disease; TOSTR, total stroke; TRSTR, thrombotic stroke; HRSTR, hemorrhagic stroke; IHD, ischemic heart disease; MI, myocardial infarction.
*Adjusted for age, sex

Appendix 13. Hwang et al. Genome-wide Manhattan plot of the meta-analysis for FPG in East Asian populations. Shown are the -log10 P values using the trend test for SNPs distributed across the entire autosomal genome (NCBI build 37). The red dots at each locus indicate the signals with P < 10−6 detected in the GWA meta-analysis. Approximately 2.4 mol/L SNPs that were present in at least 13 stage 1 studies were used to generate the plot. 2014. Diabetes



Appendix 14. Causality between fasting serum glucose and ASCVD analyzed using genetic mutations discovered in published paper

Outcome	F-statistic of WGRS(paper)	OR (95% CI)	p-value
ASCVD	1284 (n of SNP=14)	1.011 [1.000, 1.022]	0.049
AMI		1.027 [0.977, 1.079]	0.299
IHD		1.000 [0.980, 1.021]	0.985
TOSTR		1.011 [0.991, 1.030]	0.283
TRSTR		1.017 [0.988, 1.046]	0.250
HRSTR		1.009 [0.970, 1.050]	0.647

OR, Odds ratio; CI, confidence interval; FSG, fasting serum glucose; WGRS, Weighted genetic risk score; ASCVD, atherosclerotic cardiovascular disease; TOSTR, total stroke; TRSTR, thrombotic stroke; HRSTR, hemorrhagic stroke; IHD, ischemic heart disease; MI, myocardial infarction.
*Adjusted for age, sex

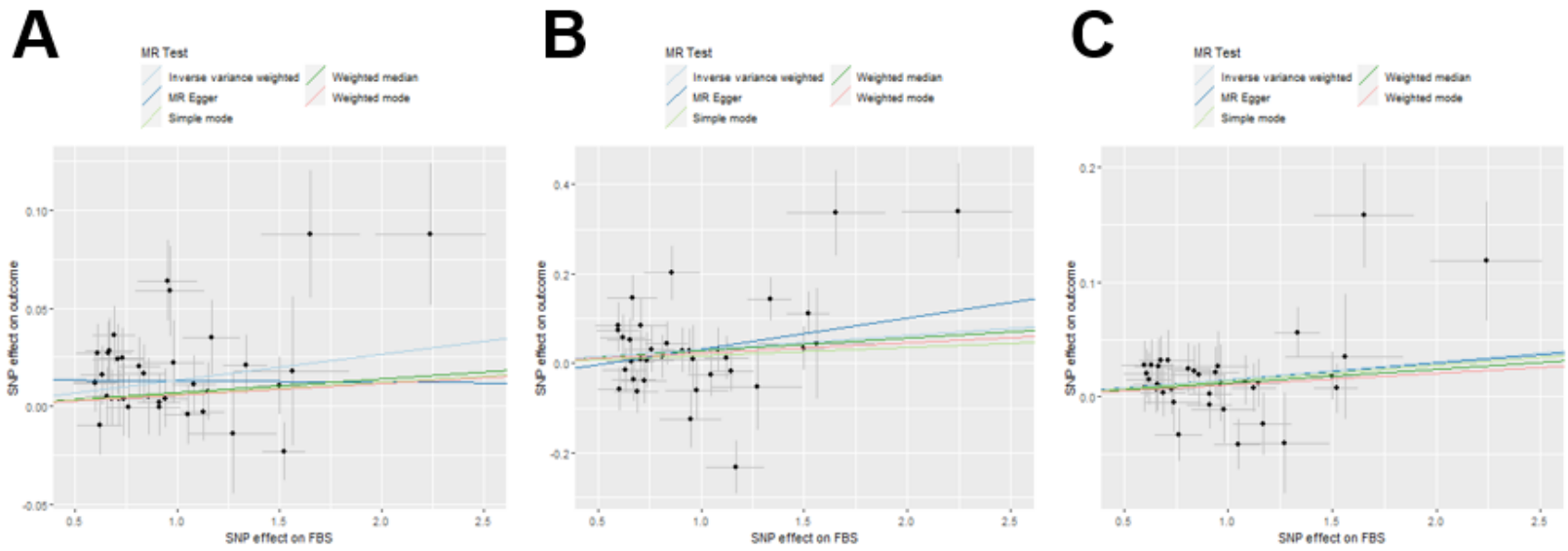
A total of 42 genome-wide significant SNPs for FSG analyzed with KoGES data in the integrated 2SMR analysis of KoGES and KCPS2 data.

no.	SNP	EA	OA	beta	se	p-value	EAF	palindromic	ambiguous
1	rs10281892	G	A	0.912077	0.105451	5.18E-18	0.4209	FALSE	FALSE
2	rs10487796	A	T	-1.04863	0.111372	4.71E-21	0.3225	TRUE	FALSE
3	rs10811662	A	G	-1.52069	0.105074	1.81E-47	0.4388	FALSE	FALSE
5	rs10908278	T	A	0.761465	0.114807	3.30E-11	0.2911	TRUE	FALSE
6	rs11065774	A	G	-1.16649	0.140862	1.22E-16	0.1647	FALSE	FALSE
7	rs11065983	C	A	0.689411	0.105895	7.50E-11	0.4299	FALSE	FALSE
8	rs111366757	A	T	0.859267	0.135354	2.18E-10	0.1842	TRUE	FALSE
9	rs111531379	C	T	2.24291	0.269681	9.03E-17	0.03875	FALSE	FALSE
11	rs114173940	T	A	1.56127	0.282475	3.26E-08	0.03546	TRUE	FALSE
12	rs11558471	G	A	-1.12593	0.105741	1.78E-26	0.4177	FALSE	FALSE
13	rs1260326	C	T	1.14811	0.105162	9.50E-28	0.4498	FALSE	FALSE
14	rs12712928	C	G	1.08286	0.107503	7.28E-24	0.3745	TRUE	FALSE
16	rs13229610	T	G	-0.811456	0.106866	3.12E-14	0.3923	FALSE	FALSE
17	rs1337919	T	G	-0.981441	0.153764	1.74E-10	0.1328	FALSE	FALSE
18	rs13387347	C	T	0.710883	0.104551	1.05E-11	0.4768	FALSE	FALSE
19	rs1574285	G	T	0.835515	0.105313	2.13E-15	0.4246	FALSE	FALSE
20	rs2241823	C	A	0.676988	0.104313	8.59E-11	0.4757	FALSE	FALSE
21	rs2497351	T	C	1.65436	0.239911	5.36E-12	0.04959	FALSE	FALSE
22	rs2525858	A	G	-0.951821	0.148457	1.44E-10	0.1446	FALSE	FALSE
23	rs35612982	C	T	1.49854	0.104153	6.17E-47	0.4658	FALSE	FALSE
24	rs3859609	T	C	-0.656165	0.106318	6.76E-10	0.3985	FALSE	FALSE

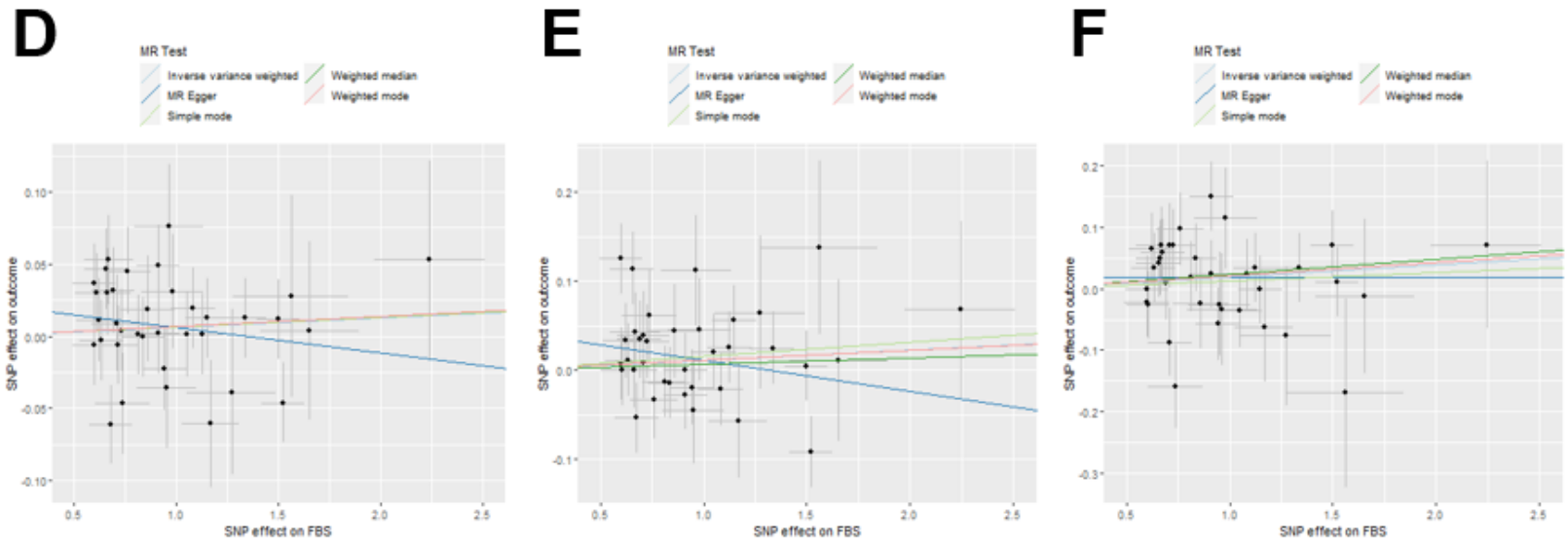
no.	SNP	EA	OA	beta	se	p-value	EAF	palindromic	ambiguous
25	rs392794	C	T	-0.607075	0.104367	6.00E-09	0.4835	FALSE	FALSE
26	rs4258054	C	T	-0.620922	0.110394	1.86E-08	0.3328	FALSE	FALSE
27	rs4728092	A	C	0.963977	0.168086	9.75E-09	0.108	FALSE	FALSE
28	rs56805921	C	G	0.633137	0.106961	3.23E-09	0.3934	TRUE	FALSE
29	rs60415045	C	A	0.943069	0.109025	5.15E-18	0.3559	FALSE	FALSE
30	rs60808706	A	G	-1.33493	0.107036	1.06E-35	0.392	FALSE	FALSE
32	rs635634	T	C	0.668475	0.11865	1.76E-08	0.2602	FALSE	FALSE
33	rs6780171	A	T	0.660475	0.115097	9.56E-09	0.2906	TRUE	FALSE
34	rs7129793	T	C	-1.27221	0.218815	6.10E-09	0.06051	FALSE	FALSE
37	rs7352806	A	G	-0.598672	0.10457	1.03E-08	0.4641	FALSE	FALSE
38	rs742761	T	C	-0.71	0.12816	3.03E-08	0.212	FALSE	FALSE
39	rs75214475	C	T	-0.598825	0.107532	2.56E-08	0.384	FALSE	FALSE
40	rs887688	T	G	-0.738169	0.133615	3.30E-08	0.1878	FALSE	FALSE
41	rs9350293	A	C	0.911404	0.107567	2.39E-17	0.3827	FALSE	FALSE
42	rs9465773	G	C	-0.727779	0.108642	2.10E-11	0.3654	TRUE	FALSE

EA, effect allele; OA, other allele; EAF, effect allele frequency

Appendix 15. Scatter plot of FSG on ASCVD

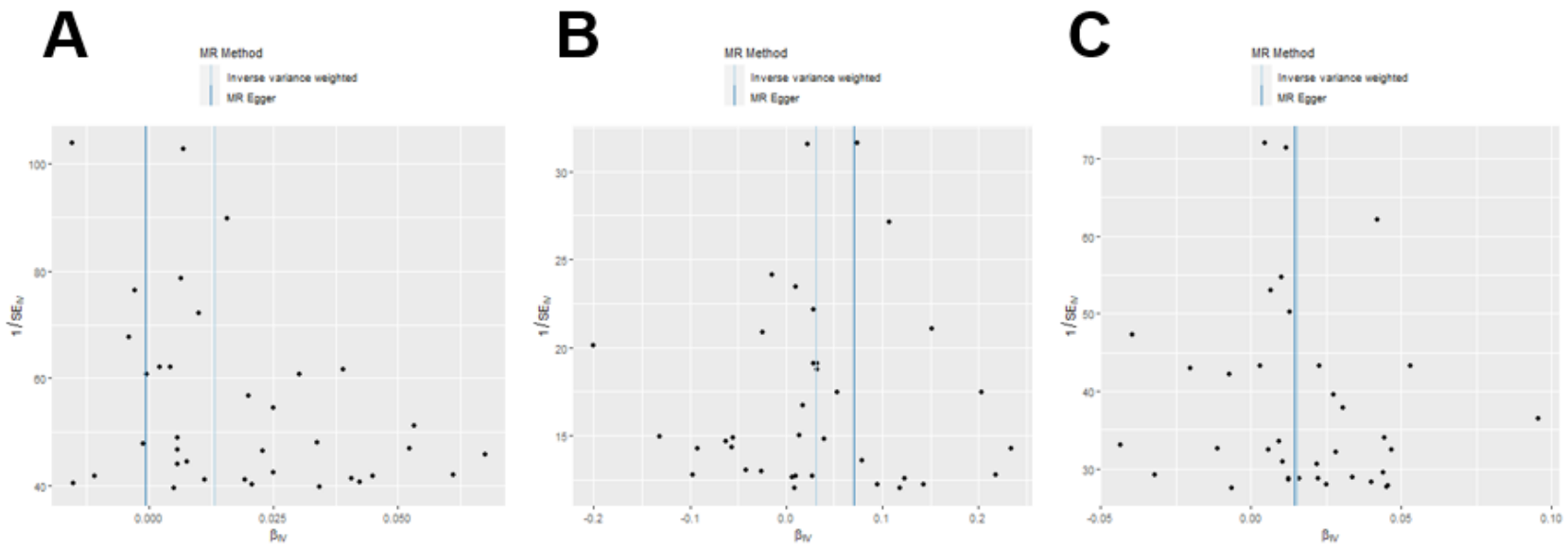


A: Atherosclerotic cardiovascular disease; B: Total stroke; C: Thrombotic stroke.

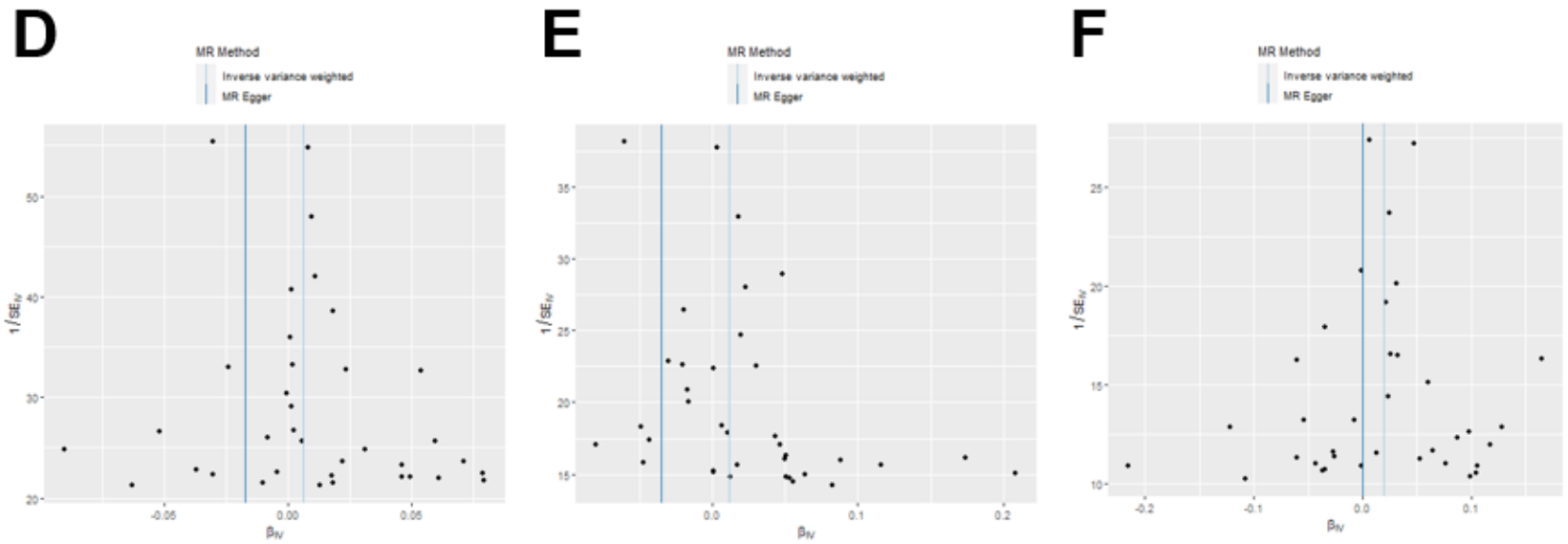


D: Hemorrhagic stroke; E: Ischemic heart disease; F: Myocardial infarction.

Appendix 16. Funnel plot of FSG on ASCVD

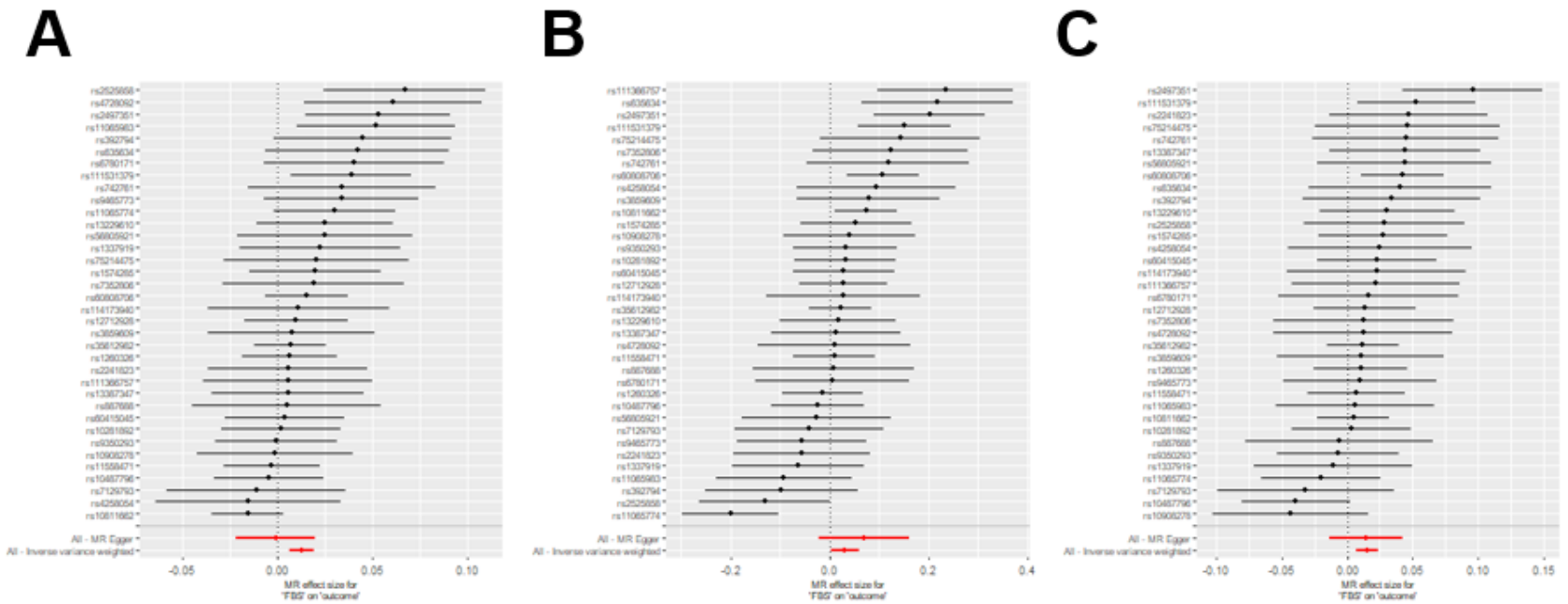


A: Atherosclerotic cardiovascular disease; B: Total stroke; C: Thrombotic stroke.

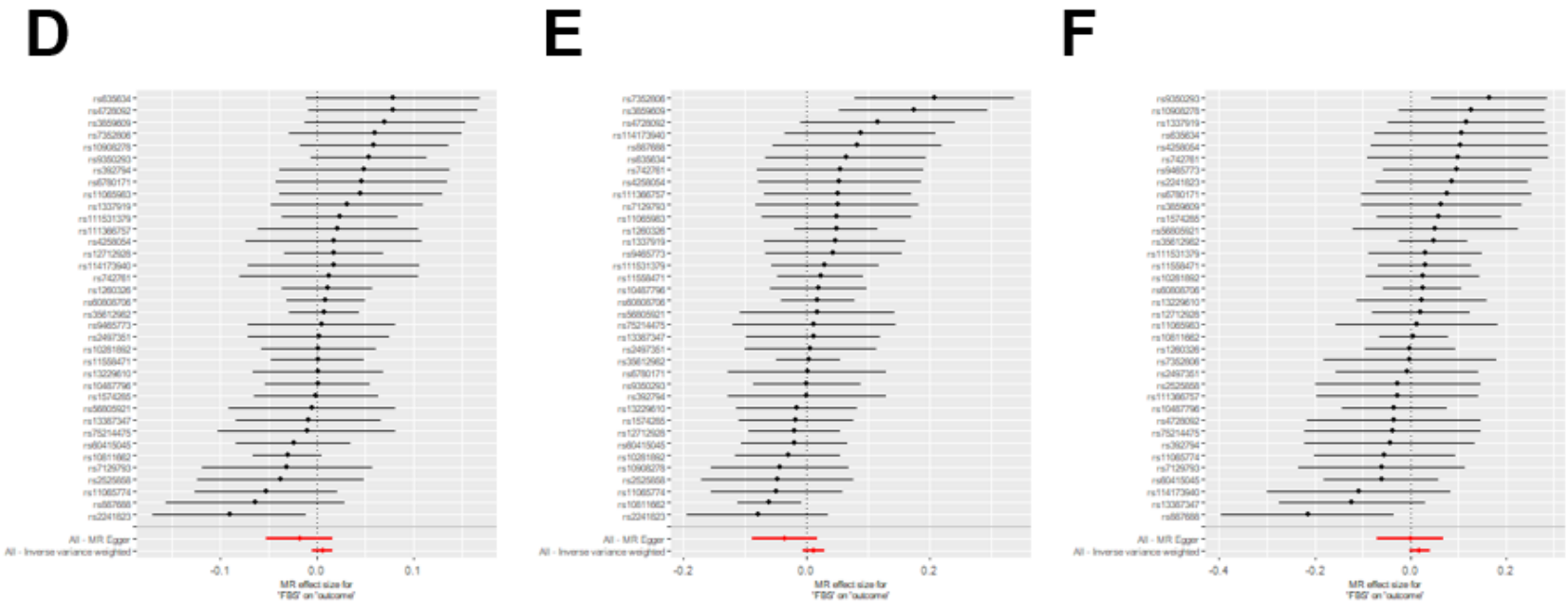


D: Hemorrhagic stroke; E: Ischemic heart disease; F: Myocardial infarction.

Appendix 17. Forest plot of single SNP MR of FSG on ASCVD

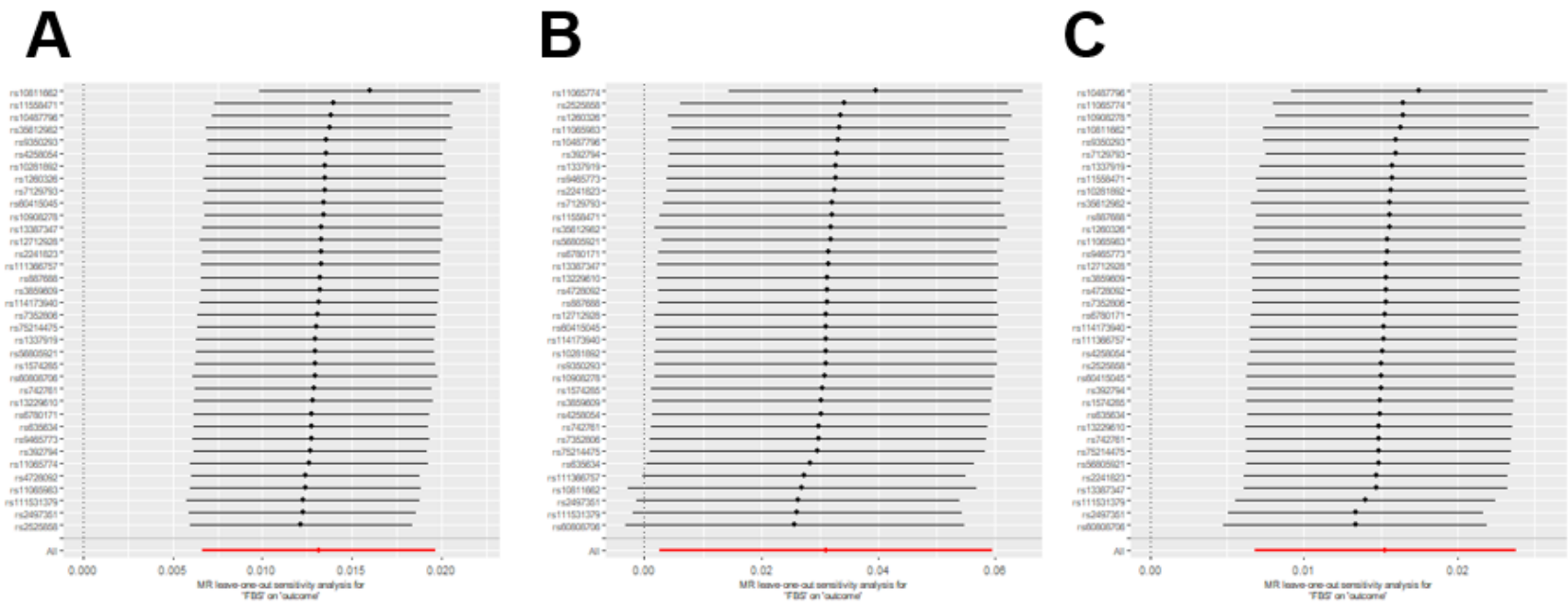


A: Atherosclerotic cardiovascular disease; B: Total stroke; C: Thrombotic stroke.

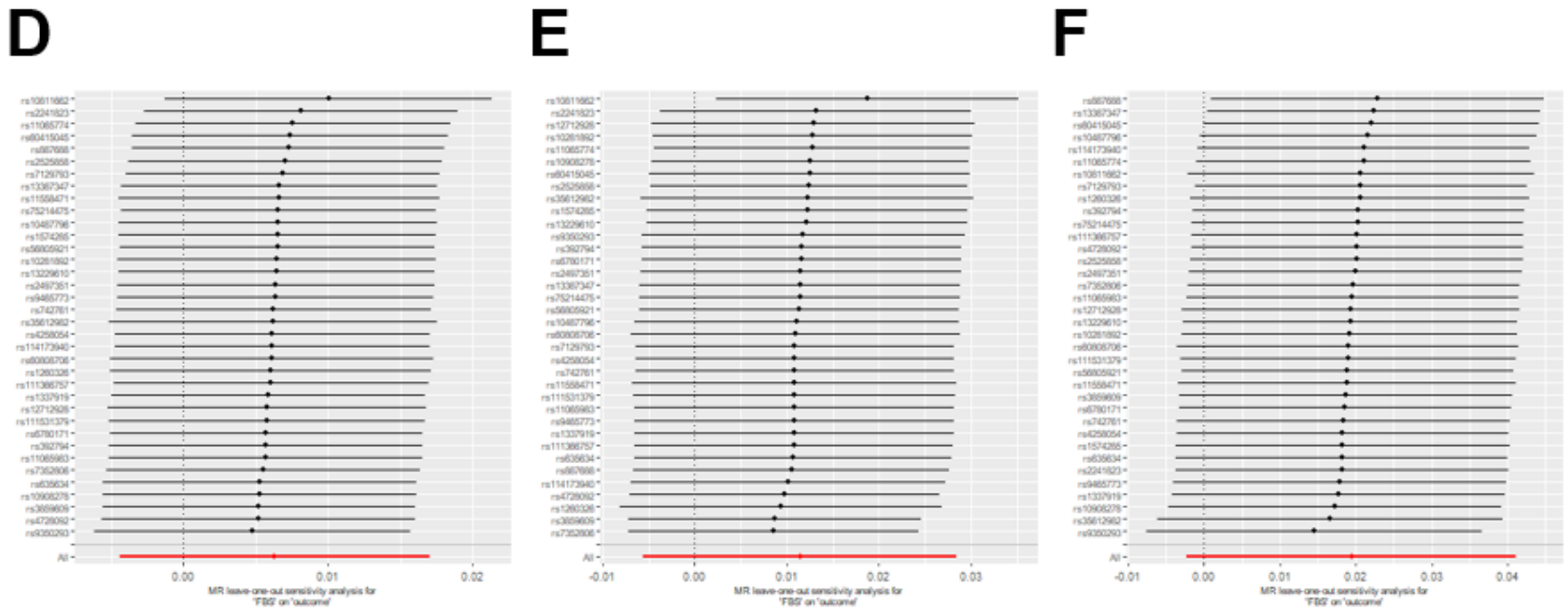


D: Hemorrhagic stroke; E: Ischemic heart disease; F: Myocardial infarction.

Appendix 18. Leave-one-out sensitivity analysis of FSG on ASCVD



A: Atherosclerotic cardiovascular disease; B: Total stroke; C: Thrombotic stroke.



D: Hemorrhagic stroke; E: Ischemic heart disease; F: Myocardial infarction.

Appendix 19. Association of fasting serum glucose with Stroke using Two-sample Mendelian randomization (KoGES, MEGASTROKE)

Exposure	Outcome	Method	Number of SNPs	Odds ratio	95% CI	P-value
FSG (KoGES)	Any stroke	Inverse variance weighted	25	1.004	0.998 -1.009	0.130
		MR Egger		0.996	0.977 -1.016	0.742
		- Intercept term test				0.436
		Weighted median		1.006	1.001 -1.012	0.032
	Any ischemic stroke	Inverse variance weighted	25	1.006	1.001 -1.012	0.032
		MR Egger		0.999	0.977 -1.022	0.932
		- Intercept term test				0.480
		Weighted median		1.010	1.003 -1.017	0.005
	Large artery stroke	Inverse variance weighted	25	1.014	0.933 -1.031	0.077
		MR Egger		0.988	0.977 -1.046	0.688
		- Intercept term test				0.354
		Weighted median		0.994	1.003 -1.031	0.177
	Cardioembolic stroke	Inverse variance weighted	25	0.998	0.986 -1.011	0.785
		MR Egger		0.994	0.951 -1.039	0.808

Exposure	Outcome	Method	Number of SNPs	Odds ratio	95% CI	P-value
		- Intercept term test				0.860
		Weighted median		0.999	0.983 -1.016	0.982
	Small vessel stroke	Inverse variance weighted	25	1.015	1.002 -1.027	0.117
		MR Egger		0.985	0.944 -1.028	0.608
		- Intercept term test				0.172
		Weighted median		1.006	0.9904 -1.021	0.18

Appendix 20. Association of fasting serum glucose with Stroke using Two-sample Mendelian randomization(KCPS-II, MEGASTROKE)

Exposure	Outcome	Method	Number of SNPs	Odds ratio	95% CI	P-value
FSG (KCPS-II)	Any stroke	Inverse variance weighted	51	1.003	0.998-1.007	0.189
		MR Egger		0.999	0.987-1.012	0.999
		- Intercept term test				0.606
		Weighted median		1.0007	0.993-1.007	0.821
	Any ischemic stroke	Inverse variance weighted	51	1.003	0.998 -1.008	0.156
		MR Egger		1.002	0.989 -1.015	0.720
		- Intercept term test				0.866
		Weighted median		1.001	0.994 - 1.008	0.657
	Large artery stroke	Inverse variance weighted	51	1.006	0.995 -1.018	0.225
		MR Egger		1.003	0.974 -1.034	0.807
		- Intercept term test				0.825
		Weighted median		1.009	0.992 - 1.027	0.286
	Cardioembolic stroke	Inverse variance weighted	51	0.996	0.986 -1.007	0.520
		MR Egger		1.002	0.974 -1.031	0.862
		- Intercept term test				0.660

Exposure	Outcome	Method	Number of SNPs	Odds ratio	95% CI	P-value
	Small vessel stroke	Weighted median		1.001	0.987 -1.015	0.828
		Inverse variance weighted	51	1.008	0.998 -1.018	0.091
		MR Egger		1.00001	0.974 -1.026	0.999
		- Intercept term test				0.501
		Weighted median		0.994	0.98 -1.007	0.393

국문요약(Korean Abstract)

공복혈당이 죽상동맥경화성 심혈관질환에 미치는 인과적 영향에 대한 삼각측량(Triangulation) 연구

연세대학교 보건학과

이수현

연구배경: 당뇨병은 죽상동맥경화성 심혈관질환(ASCVD)의 위험을 증가시키는 위험인자로 알려져 있으나, 이전의 관찰연구에서는 이상혈당증 관련 지표와 심혈관질환 사이의 인과관계를 확립하지 못했다. 최근 역학 연구에서는 인과 추론의 한계를 개선할 수 있는 과학적 연구 방법으로 삼각측량법 (Triangulation)이 제안되고 있다. 본 연구의 목적은 삼각측량법을 이용하여 공복혈당과 심혈관질환의 인과관계를 확인하는 것이었다.

연구방법: 본 연구에서는 임상시험의 메타분석, 코호트 분석, 멘델의 무작위화(MR) 분석을 이용하여 삼각측량법을 분석하였다. 메타분석은 임상시험 (RCT)와 관찰연구인 코호트 연구를 각각 수행하였다. 코호트 분석은 한국인 암 예방연구-II 자료를 활용하였다. 한국인 암 예방연구 (KCPS)-II 대상자는 2004-2013년 서울, 경기를 포함하여 전국에서 18개 종합건강검진센터를 방문한 159,844명을 대상으

로 연구참여 동의서를 획득하고 구축한 혈액기반 전향적 코호트 연구이다. 멘델의 무작위화(MR)분석은 공복혈당은 한국인 유전체 역학연구 (KOGES) 바이오 뱅크 자료를 사용하였고, 죽상동맥경화성 심혈관질환 발생은 한국인 암 예방연구에서 확인된 자료를 활용하였다. 부가적으로 죽상동맥경화성 심혈관질환 자료는 일본의 바이오 뱅크 (BBJ)도 활용하였다. 멘델의 무작위화(MR)분석은 한국인 암 예방연구-II 내에서 표준 (one-sample) MR, KOGES와 KCPS-II 바이오 뱅크를 활용하여 two-sample MR, 그리고 수축기 혈압과 LDL 콜레스테롤의 유전변수를 통제한 다변수 MR (MVMR)를 각각 분석하였다. 이 연구에서 공복혈당은 10mg/dL 증가당 영향을 보았다.

연구결과: 삼각측량법의 결과 모든 연구에서 공복 혈당이 ASCVD에 영향을 미치는 것으로 나타났다. 첫째, 임상시험의 메타분석 결과는 당뇨병 치료군에 비해 중재군에서 심혈관 질환 위험이 17% 낮은 것으로 나타났다. 둘째, 관찰연구로서 코호트 연구의 메타분석 결과는 공복혈당 10mg/dL 증가 마다 심뇌혈관 질환 위험이 3.7% 증가하는 것으로 나타났다. 셋째, 코호트 연구에서는 공복 혈당 수치가 한 단위 증가할 때마다 ASCVD 위험이 4% 증가하는 것으로 보였다(HR=1.049, 95% CI=1.039-1.059). 넷째, 표준 MR (one sample MR) 연구 결과에서도 공복혈당 수치가 10mg/dL 증가마다 ASCVD 발생을 13.4% 증가시키는 것으로 나타났다(HR=1.134, 95%CI=1.038-1.238). 다섯째, 2-표본 MR 결과 ASCVD에서

공복 혈당 10 mg/dL 증가당 OR은 1.110으로 통계적으로 유의한 것으로 나타났다(95% CI=1.110-1.180). 마지막으로 다변수 MR 에서는 공복 혈당 10 mg/dL 증가당 ASCVD를 13.8% 증가시키는 것으로 나타났다 (OR=1.138, 95%CI=1.068-1.214).

결론: 공복혈당은 한국인의 ASCVD 발생과 인과관계가 있는 것으로 나타났다. 따라서 본 연구 결과는 혈당 조절이 심혈관 질환의 평생 위험을 실질적으로 줄일 수 있는 잠재력이 있음을 시사한다.

핵심어: 공복혈당, 죽상동맥경화성 심혈관질환(ASCVD), 삼각측량법