Ph.D. Dissertation of Public Health

A comprehensive evaluation for type-2 diabetes using genome-wide association studies and Mendelian randomization

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A comprehensive evaluation for type-2 diabetes using genome-wide association studies and Mendelian randomization

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Abstract

Background: As the rapid development of advanced genotyping technologies has led to an explosion of genetic data, genome-wide association studies on type 2 diabetes have identified more than 400 distinct genetic loci associated with diabetes and nearly 120 loci for fasting glucose and fasting insulin level. However, genetic risk factors for the longitudinal deterioration of fasting glucose have not been thoroughly evaluated. Meanwhile, multiple investigation has demonstrated that type 2 diabetes (T2D) affect several cancers and vascular disease. In addition, T2D has been reported to be associated with severe mental illness (SMI) such as schizophrenia (SCZ), bipolar disorder (BPD), and major depressive disorder (MDD). However, most of them were based on observational epidemiological studies which suffer from many potential biases, confounding and reverse causation.

Objective: The aim of this study is (1) to identify genetic variants associated with longitudinal change of fasting glucose over time, (2) to identify major cancers and vascular diseases which can be affected by T2D related traits, fasting plasma glucose (FPG) levels, 2-hour postload glucose (2h PG), and HbA1c, by using Mendelian randomization (MR) study (3) to identify the causality between T2D and SMI using MR study.

Methods: To investigate the impact of genetic variants on fasting glucose and its longitudinal change, we used two prospective cohort studies including individuals

without type 2 diabetes at baseline examination and their genotypes. Linear mixed model analysis was performed, and the results of the two cohorts were meta-analyzed using METAL software. To investigate causal effect of T2D related effects on various human health phenotype, the summary statistics for FG, 2h PG and HbA1c were obtained through large-scale genome-wide association meta-analyses of 133,010 non-diabetic individuals from the collaborating studies within the Meta-Analysis of Glucose and Insulin related traits Consortium (MAGIC). We obtained summary genetic associations with a total of 70 human health phenotypes through MR-BASE platform. This registry comprises GWAS summary data including over 11 billion genetic variants related with various phenotypes from 1673 GWAS. We conducted five MR analyses (Inverse-variance weighted method, MR-Egger, MR-Egger with with a simulation extrapolation (SIMEX), MR-PRESSO and weighted median method) to identify traits affected by FPG, 2h PG and HbAlc. For SMI and T2D, summary data were obtained from Psychiatric Genomics Consortium and DIAbetes Genetics Replication And Meta-analysis Consortium identify the causality between them.

Results and Conclusions: One variant, rs11187850 in intron of *PLCE1* (*phospholipase C epsilon 1*), achieved genome-wide significant association for longitudinal change in fasting glucose ($P = 4.85\times10^{-08}$) and three variants had suggestive evidence for an association ($P < 1.00\times10^{-05}$): rs10947494 ($P = 3.64\times10^{-06}$) near *NUDT3* (*nucleoside diphosphate linked moiety X-type motif 3*), rs2414772 ($P = 6.30\times10^{-06}$) near *MIR6085* (*MicroRNA 6085*), and rs16959641 ($P = 2.64\times10^{-06}$) near *USB1* (*U6 snRNA biogenesis phosphodiesterase 1*). In MR analyses, we found

that coronary artery disease was affected by higher FPG, but bipolar disorder is decreased by higher FPG. Furthermore, LDL cholesterol have causal relationship with HbA1c. Furthermore, we found that MDD had a significant causal effect on T2D. The absence of reverse-causality between all significant diseases was also demonstrated from bi-directional MR studies.

Keyword: Genome-Wide Association Study (GWAS), Longitudinal data analysis, Mendelian Randomization (MR), type 2 diabetes, fasting plasma glucose (FPG), 2-hour postload glucose (2h PG), HbA1c, bipolar disorder (BPD), major depressive disorder (MDD), schizophrenia (SCZ)

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Introduction

1. Study Background

Prediabetes, which is usually defined as a blood glucose that is higher than normal but lower than the diabetes threshold, has a higher risk of developing diabetes. According to the American Diabetes Association (ADA), the criteria for blood glucose metabolism can be classified as follows: (1) normal glucose tolerance, NGT (fasting plasma glucose (FPG) < 100 mg/dL) (2) impaired fasting glucose, IFG (FPG 100 ~ 125 mg/dL); (3) impaired glucose tolerance, IGT (2h plasma glucose (2h PG) 140 ~ 199 mg/dL) (4) T2D (FPG ≥ 126 mg/dL or 2h PG ≥ 200 mg/dL). IFG and IGT considered as prediabetes. Every year, 5-10% of pre-diabetes patients develop diabetes, and the same percentage is converted to normal glucose level. The term prediabetes has been criticized because many people with prediabetes do not progress to diabetes, and may not require intervention because there is no disease. However, prevalence of prediabetes is increasing across the world and experts expected that more than 470 million people will have prediabetes by 2030 [1]. Furthermore, important difference is that the level of hyperglycemia is different from the normal level of risk of developing complications associated with diabetes. Hyperglycemia level is a stage that receives a lot of attention to prevent diabetes because it corresponds to a high risk of developing T2D. IFG and IGT are known to have 5 to 17 times higher risk of developing T2D [2-4]. Therefore, it has an important meaning as a preclinical stage that must be actively intervened before progressing to diabetes.

Trajectories of glycemic changes in prediabetes

Based on a prospective cohort study, a total of 6,538 (71% male and 91% white) British civil servants without diabetes mellitus at baseline were participated in the British Whitehall II study [5]. Participants were then traced backwards to their first clinical screening. Multilevel longitudinal modelling was used to estimate trajectories of fasting glucose in diabetics before diagnosis and in non-diabetics before last screening. The results show that glucose levels increased at the beginning of 13 years prior to diagnosis in the diabetes patients, but glucose levels appeared to be tightly controlled until 2-6 years before diagnosis within the normal range [1]. Other studies have confirmed the similar pattern of glycaemic changes, which is a rapid increase in fasting glucose several years before diagnosis of diabetes [6, 7]. The cause of early fasting glucose fluctuations can be caused by environmental and lifestyle factors. However, the exact biological mechanisms that support why people progress differently to hyperglycemia are unclear.

Genetic risk factor for fasting glucose

The fasting glucose levels are moderately hereditary, with the heritability around 30%. A number of genetic determinants that affect FPG have been identified in numerous genome-wide association studies (GWAS) in the past few years [8, 9]. To date, meta-analysis performed in MAGIC (the Meta-Analysis of Glucose and Insulin traits Consortium) identified genetic loci associated with FPG in non-diabetic European individuals and show the discovery of multiple associated 39 loci [10]. Furthermore, most of them (23 loci) were detected mostly in the GWAS of East Asian (EA) populations, but others were not replicated in EA individuals and identified

additional loci significantly associated with FPG [9]. However, previous GWAS of fasting glucose have typically restricted the study output to include only single measurement of traits or average measures of repeated measurements. Although there have been several studies that investigated the genetic association for longitudinal change in fasting glucose, there has not been a locus that reached genome-wide significance [11, 12].

Environmental risk factor for fasting glucose

fasting glucose level increased as age increases, at a rate of 12.6 – 19.8 mg/dl per age decade [13]. Furthermore, the elevation in plasma glucose associated with age are to a certain extent due to age-related environmental factors. There might be individual variation in the rate of longitudinal glucose change affected by several environmental factors which are difficult to control. In general, fat increases and muscle mass is reduced as individuals become old. It is estimated, with age, that muscle reduction is related to abnormal functioning of mitochondria and increase in fasting glucose is caused by insulin resistance [14].

Public health implications for type 2 diabetes and human health disease

Prediabetes are not only associated with an increased risk of diabetes and its complications, but can also damage the kidneys and nerves based on accumulated evidence. Several studies have found a link between increased risk of chronic kidney disease and early nephropathy with diabetes [15, 16]. The cause of this relationship is unclear because this relationship may be due to the increased incidence of diabetes in this group or the presence of other factors associated with hyperglycemia and

nephropathy rather than the effects of prediabetes [17, 18]. Furthermore, prediabetes have been found to be associated with dysfunction of cardiac autonomic activity reflected by a decrease in heart rate changes [19, 20] and associated with increased risk of developing macrovascular disease but whether this elevated risk is due to prediabetes itself or due to development of diabetes remains unclear [21]. While a cross-sectional study showed an increased prevalence of coronary heart disease in prediabetes patients [22]. However, this relationship can be confounded by the common risk factors that exist between cardiovascular disease and pre-diabetes.

Severe mental disease and type 2 diabetes

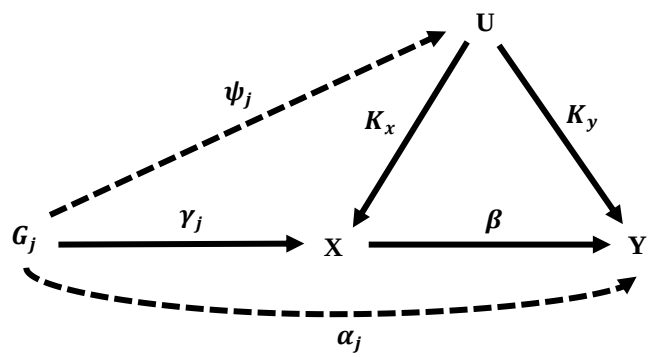
The prevalence of type 2 diabetes among individuals with SMI has been estimated to be 8% to 17% in BPD, and 16% to 25% in SCZ; further, depressed adults have a 37% increased risk of developing type 2 diabetes [39-41]. The side effects of the medication used for treating SMI, unhealthy lifestyle behaviors of patients with SMI, and hypothalamic-pituitary-adrenal (HPA) axis dysregulation could contribute to the association of SMI with type 2 diabetes [42, 43]. For instance, medications such as antipsychotics, antidepressants, and mood stabilizers are likely to contribute to type 2 diabetes development by leading to insulin resistance or weight gain [44]. Moreover, low physical activity, poor diet, smoking, alcohol, and substance abuse in individuals with SMI might lead to type 2 diabetes [45]. In contrast, type 2 diabetes also affects mental health. One meta-analysis indicated that the risk of MDD increases in people with type 2 diabetes [46], and a prospective population-based study showed that the prevalence of schizophrenia was significantly higher in patients with type 2 diabetes than in the general population

[47]. Further, a cross-sectional study reported that type 2 diabetes and prediabetes may be risk factors in patients with BPD [48]. Large longitudinal studies have also demonstrated that type 2 diabetes impacts mental health negatively [49].

Mendelian randomization study: assumptions and methods

MR Assumptions

The assumptions of MR studies can be represented using causal directed acyclic graph (DAG):



In the DAG, the genetic variant G_j (j=1, 2, …, J) and the exposure X is denoted γ_j, and the association between genetic variant G_j and the outcome Y is denoted α_j. Association between confounding factor U and G_j, X and Y are denoted ψ_j, K_x and K_y, respectively. In a two-sample MR setting, we refer to $\hat{\gamma}_j$ as an estimate from jth SNP – exposure association (with variance $\sigma^2_{X_j}$) from the sample 1 and $\hat{\alpha}_j$ as an estimate from jth SNP – outcome association (with variance $\sigma^2_{Y_j}$) from the sample 2.

$$\text{Sample 1: } \hat{\gamma}_j = \gamma_j + k_x\varphi_j + \epsilon_{X_j},\ \text{var}\left(\epsilon_{X_j}\right) = \sigma^2_{X_j}$$

$$\text{Sample 2: } \hat{\alpha}_j = \alpha_j + k_y\varphi_j + \beta\left(\gamma_j + k_x\varphi_j\right) + \epsilon_{Y_j},\ \text{var}\left(\epsilon_{Y_j}\right) = \sigma^2_{Y_j}$$

The genetic variants G_j for valid instrumental variables must be satisfied following three core assumptions (IV1, IV2, IV3).

IV1: $\gamma_j \neq 0$ (strongly associated with intermediate exposure)

IV2: $\varphi_j = 0$ (IVs independent of confounders)

IV3: $\alpha_j = 0$ (IVs affect the outcome only through the exposure path)

Additionally, MR requires the "NO Measurement Error" (NOME) assumption and the InSIDE assumption (INstrument Strength Independent of Direct Effect). The former means that the SNP-exposure associations are estimated without measurement error, $\sigma_{X_j}^2 = 0$ (negligible uncertainty in SNP-exposure association) and the latter assumes $\mathrm{cov}(\alpha_j, \gamma_j) = 0$.

Assessing the instrument strength is important to avoid weak instrument bias in MR analysis. The weak instruments can be reliably detected using the mean F-statistics and the degree of violation of the NOME assumption can be quantified using the I^2 statistic, a number ranging between 0 and 1. Higher values for I^2 indicating less dilution of the causal effect estimate.

The F-statistics for variant j can be approximated as

$$F_j = \hat{\gamma}_j^2 / {\sigma^2}_{X_j}.$$

J Bowden at el. [23] defined the I^2 statistic as follows:

$$I^2 = \frac{\mathrm{Q} - (L-1)}{\mathrm{Q}}$$

where a Cochran's Q statistic, $\mathrm{Q} = \sum_{j=1}^{L} \frac{(\hat{\gamma}_j - \bar{\hat{\gamma}})^2}{{\sigma^2}_{X_j}}$ and $\bar{\hat{\gamma}}$ means the mean of the SNPs-exposure associations weighted by ${\sigma^2}_{X_j}$.

MR Methods

Inverse-Variance Weighted method

With all genetic variant G_j that satisfies the three IV assumptions, NOME and InSIDE assumptions, the causal effect of the exposure on the outcome can be consistently estimated from the ratio estimates through each genetic variant can be averaged using an inverse-variance weighted (IVW) method [24]. If the genetic variants are uncorrelated (not in linkage disequilibrium) the ratio estimates can be obtained as

$$\hat{\beta}_{\text{IVW}} = \frac{\sum_j \hat{\gamma}_j^2 \sigma_{Y_j}^{-2} \hat{\beta}_j}{\sum_j \hat{\gamma}_j^2 \sigma_{Y_j}^{-2}}.$$

The IVW estimate is an efficient method when all genetic variants are satisfied all three IV assumptions. Unfortunately, the estimate will be biased even if only one variant is invalid, we consider sensitivity analysis to minimize bias.

Weighted Median Method

In contrast to the IVW estimate, weighted median method provide valid causal estimates even if up to 50% of the instruments are invalid. The median is not affected by outliers, and so the weighted median estimate is not sensitive to a pleiotropic genetic variant. Causal effects obtained from the weighted median of the ratio estimates in genetic instruments which smaller standard error receive more weight. Moreover, the weighted median estimator is consistent because unlike the IVW estimate taking a weighted mean of the ratio estimates (α_j/γ_j), this method used the median of the ratio estimates [25].

MR-Egger and MR-Egger with simulation extrapolation

MR-Egger method is also sensitivity method allow that all SNPs is invalid instruments but requires variants to satisfy a weaker assumption, the InSIDE assumption, and then it can estimate appropriate causal effects in the presence of pleiotropy effects [26]. This model set the intercept term not to be zero, which is estimated as part of the analysis:

$$\hat{\beta}_{\text{Egger}} = \beta_{0E} + \beta_E \hat{\gamma}_j.$$

The intercept term β_{0E} can be interpreted as the average horizontal pleiotropic effect across the genetic variants [26]. It regresses α_j on γ_j assuming that the InSIDE assumption holds, then the slope β_E of the MR-Egger regression provides a causal effect. If estimates of β_{0E} equal to zero, then MR-Egger slope estimate will be same as the IVW estimate [27]. However, when the I^2 statistic quantifying the strength of NOME violation for IVs for MR-Egger method is low, the magnitude of regression dilution sill occur. In cases where the violation of the NOME assumption, the method of simulation extrapolation (SIMEX) can be used as methods of correcting for attenuation bias [23]. Under SIMEX, new data sets are generated from simulating for SNP-exposure association estimates under increasing violations of the NOME assumption ((1+λ) times as large as $\sigma^2{}_{X_j}$, where λ is a non-negative number). Combined data sets with new simulation data were used to yield a new estimate for $\hat{\beta}_E$. A statistical model is then fitted to the set of causal estimates obtained across the λs and extrapolating back to the $1 + \lambda = 0$ for bias-adjusted inference.

Mendelian Randomization Pleiotropy Residual Sum and Outlier Test

Violation of the IV3 (no horizontal pleiotropy) can raise a severe bias in MR study. The Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO) test has the advantage over MR-Egger of identifying and removing pleiotropic SNPs. The test consists of three parts, (1) the MR-PRESSO global test which detects horizontal pleiotropy, (2) the outlier corrected causal estimate which corrects for the detected horizontal pleiotropy and (3) the MR PRESSO distortion test which estimates if the causal estimate is significantly different (at $p<0.05$) after adjustment for outliers [28].

2. Literature Review

Previous Genome-Wide Association Study for Longitudinal Change in Fasting Glucose

A genome-wide association meta-analysis with non-diabetic European ancestry individuals [11] is a largest study for fasting glucose change over time. In this study, a total of 13,807 participants consisted of nine cohorts of European descent representing three continents (America, Europe and Australia) and they were repeatedly measured at two or more time points up to 14 years. The associations of genetic variants with inverse-normal transformed fasting glucose change over time were studied adjusting for baseline-age, sex and principle components of genetic variation. However, they found no genome-wide significant association ($<5 \times 10^{-08}$) with fasting glucose over time but suggest three suggestive associated variants (near *ODZ4, ALLC*, and *NUDT12*). Furthermore, the other European GWAS regarding to FPG trajectories, there no genetic variants associated with glucose deterioration over time achieved genome-wide significant threshold [29]. However, in a Han Chinese GWAS, they showed the increase in FPG over time is greater compared to those with fewer T2D risk-increasing variants by GWAS replicate [30]. In the GWAS using the population-based Atherosclerosis Risk In Communities (AIRIC), five genomic regions significantly associated to the longitudinal change of FPG (*GCKR, G6PC2, GCK, SLC30A8, MTNBAB*) were suggested [31]. However, this study included diabetic patients taking medications that could act as a confounding factor to associations of genotypes and change of FPG.

Type 2 Diabetes Related Traits Effect on cancers and vascular diseases

Chronic hyperglycemia, as assessed by FPG, 2h PG, and HbA1c levels, has been the main risk factor for the development of diabetes-related complications. Many epidemiological (prospective and retrospective) studies have been reported the association between glucose variability and diabetes-related complications. Cardiovascular disease are the major causes of morbidity and cardiovascular death in patients with T2D patients [32]. A positive association between increased variability and microvascular complications and coronary artery disease was consistently reported [33]. Several recent researches have investigated the association between variability in HbA1c and micro- and macrovascular complications. As for macrovascular complications, FPG has been independently associated with cardiovascular death and heart failure, coronary artery disease, stroke and all-cause mortality [34]. A number of studies have suggested that variability in HbA1c may be an important factor in the development of nephropathy [35, 36]. Furthermore, significant link between blood glucose and retinopathy or nerve conduction abnormalities were reported [37]. A number of cancers such as pancreatic, liver, breast, and female reproductive cancers have shown an increased prevalence and a higher mortality rate in diabetic patients compared to healthy individuals [38]. In addition, several studies showed that the relationship of depression and glycemic control. Previous randomized controlled trial (RCT) studies assessed treatment-related improvement in glycemic control were directly associated in relieving depression [39, 40]. The meta-analysis confirms the association of depression with hyperglycemia but reveals neither the mechanism nor the direction of the association [41].

Causal relationship between severe mental disease and type 2 diabetes

MR studies on the association between SMI and type 2 diabetes are scarce, with no studies on BPD with type 2 diabetes. To investigate the potential causal relationship of type 2 diabetes with MDD, MR analysis was performed with a large longitudinal cohort from 2011 to 2013 in China [42]. Genetic risk scores for type 2 diabetes were chosen as the instruments and two-stage multiple regression was used for statistical analysis. The results provided evidence of a potential causal effect of type 2 diabetes on MDD, which is the opposite of our results. We thought that there is a finite-sample bias in the existing research, because in a one-sample setting, the fitted values from the first-stage regression are correlated with the outcome in finite samples even in the absence of a causal effect [43]. Regarding the MR studies of SCZ and type 2 diabetes, two-sample MR was performed using the IVW and MR-Egger methods in Europeans, East Asians, and trans-ancestry groups [44]. No evidence of a causal role of type 2 diabetes for SCZ was observed in any of the analyses, which is consistent with our findings; however, they did not perform bi-directional analysis for causal effect of SCZ on type 2 diabetes. Unlike epidemiological studies, the previous and the present MR studies could not consider multi-episode status of disease, which may have led to non-significant results of SCZ and BPD.

3. Purpose of Research

The purpose of this research is as follows;

1) Identifying genetic variants associated with longitudinal change of fasting glucose over time using two large-scale prospective cohorts

2) Verifying causal relevance of T2D to a various human phenotype through Mendelian randomization (MR) study using the publicly large-scale GWAS data from MR-BASE platform.

3) Identifying the causality between T2D and SMI using MR study from Psychiatric Genomics Consortium (PGC) and DIAbetes Genetics Replication And Meta-analysis Consortium (DIAGRAM).

PART I. Genome-Wide Association Study on Longitudinal Change in Fasting Glucose

1.1. Research Problem

Type 2 diabetes is diagnosed based on discrete values of plasma glucose and Hba1c. However, dysglycemia is a continuum of abnormal glucose level that is above normal glucose level and include prediabetes and diabetes. There has been a number of studies that investigated the natural course of in the development of type 2 diabetes [5, 45-47]. Based on a prospective cohort specifically designed to study the trajectories of dysglyemia, those who progressed to diabetes had already elevated fasting glucose level 10 years before the development of diabetes compared to those who remained normal glucose tolerance [5]. There seems to be a rapid increase in fasting glucose 2 - 6 years before diagnosis of diabetes [5, 45]. In addition, fasting glucose level increased as age increases, at a rate of 12.6 – 19.8 mg/dl per age decade [13]. However, there might be individual variation in the rate of longitudinal glucose change. This is also suggested by the fact that only about 25% of prediabetes subjects progress to type 2 diabetes in five years of follow-up [48]. The genetic contribution on this longitudinal change in fasting glucose level has not been well investigated thoroughly.

Genome-wide association studies (GWAS) on type 2 diabetes have identified more than 400 distinct genetic loci associated with diabetes [49, 50] and nearly 120 loci for fasting glucose and fasting insulin level [8]. However, it should be noted that genetic loci for fasting glucose and type 2 diabetes does not overlap completely [8]. This discrepancy could be due to multiple factors including different

range of glycemia studied for fasting glucose and type 2 diabetes, interaction by environmental factors, and most of the studies being cross sectional in nature. Although there have been several studies that investigated the genetic association for longitudinal change in fasting glucose [51, 52], there has not been a locus that reached genome-wide significance.

Therefore, the main interest lies in dependence of the evolution of the outcome over time on the SNP alleles, the p-value of the SNP × time interaction is of central importance with a longitudinal design. To this end, there are two popular methods to deal with repeated measurements: linear mixed model (LMM) and generalized estimating equations (GEE). LMM allows for correlation within subject to vary by a specific pattern which produces models that better fit the data. Additionally, subjects with missing outcome and different numbers of visits can be included as long as the time intervals are correctly specified in the model. Because LMM uses maximum likelihood estimation (MLE), it is robust against missing at random (MAR) data [53]. GEE has the same advantages as LMM in that it allows correlation structures through working correlation matrix and it can take several types of covariates. However, the main difference is that GEE only provides population average estimates not individual level information for random effects. Since it requires complete data or missing completely at random (MCAR) as it is not likelihood based, it is less robust than LMM in missing data scenarios [53, 54].

In summary, we hypothesized that using LMM will allow us to investigate the impact of genetic variants on fasting glucose and its longitudinal change in an East Asian population. For this end, we used two large-scale prospective cohorts, Korea Genome and Epidemiology Study (KoGES) and Gene-Environment

Interaction and phenotype (GENIE) cohort, to perform GWAS using LMM and meta-analyzed the results.

1.2. Materials and Methods

Study participants

The KoGES [55] was initiated in 2002, and 6,122 individuals who did not have type 2 diabetes at baseline examination were investigated [5]. The participants consisted of 2,847 males and 3,275 females, with age at baseline range of 40 – 69 years. Fasting glucose and post-challenge 2-h plasma glucose were measured every 2 years from 2001 to 2012. The diagnosis of diabetes was defined according to the American Diabetes Association criteria: fasting glucose ≥ 126 mg/dL; post-challenge 2-h plasma glucose ≥ 200 mg/dL after a 75-g oral glucose load; and HbA1c ≥ 6.5%. The subjects were followed up for 11 years on average, and the minimum and maximum follow-up time were 6 and 12 years, respectively. During the follow-up period, 790 participants developed incident type 2 diabetes, and their fasting glucose levels after the first diagnosis of diabetes were not included in the analyses.

The GENIE cohort consisted of 7,999 participants who had visited Seoul National University Gangnam Center between during 2014. A total of 4,406 participants (2,604 males and 1,802 females) who did not have type 2 diabetes at their baseline visit were considered for inclusion in the present analyses. The number of visits to the Gangnam Center varied among the participants, and those who visited the center more than three times, but less than 13 times were ultimately including in our analysis. The participants were followed up for 6 years on average. Type 2

diabetes was diagnosed according to the ADA definition as described above. During the follow-up period, 237 participants developed incident type 2 diabetes and their fasting glucose values after the first incidence of diabetes were excluded.

Genotyping and imputation

Genome-wide genotyping on the KoGES and GENIE cohorts was performed using the Affymetrix Genome-Wide Human SNP Array 5.0 and Affymetrix KOR_v1.0, respectively. We excluded any single nucleotide polymorphism (SNP) marker with more than 5% missingness, minor allele frequency (MAF) < 0.05, Hardy-Weinberg equilibrium p-values < 1.0×10^{-06}, and any subjects with more than 5% missing genotype calls and sex inconsistency. Imputation was conducted with IMPUTE2 using the cosmopolitan reference panel from 1000 Genomes Project phase 3. Any imputed SNPs with imputation quality scores < 0.4 were excluded. After quality control, 3,758,649 and 3,692,736 SNPs were used for association analyses of longitudinal change in fasting glucose in the KoGES cohorts and GENIE cohorts, respectively. The variant annotation was performed by ANNOVAR [56]. Genotype dosage scores obtained from IMPUTE2 were used for association testing [57].

Genome-Wide Association Analyses

Association analyses were performed with an LMM using the lme4 package in R v.3.4.1 [58] with two random effects, intercept and slope over time, for each subject and correlations between two random effects were allowed. We considered several models and the best model was selected by Aikaike's information criterion. We applied a number of different kinds of model to the analysis according to the following criterions: (1) how to assume the random model (random intercept model/ random intercept and slope model) (2) If both slope and intercept exist, whether to allow correlations between two random effects, intercept and slope over time, for each subject; (3) whether to consider the inverse variance of each time point dataset as weights (Table 1.1). The selected model has two random effects for intercept and slope over time for each subject, with nonzero correlations between them. SNP, sex, baseline age, elapsed time on FPG, the 10 principal component (PC) scores, and the SNP-by-time interaction were included as covariates. As body mass index (BMI) is a major risk factor for T2D and is correlated with FPG, we also performed two kinds of analyses for BMI-adjusted and -unadjusted model. For the *j*-th measurement of the *i*-th subject, our LMM for both the KARE and GENIE cohorts can be expressed by

$$FG_{ij} = \beta_0 + \beta_1 age_i + \beta_2 sex_i + \beta_3 BMI_{ij} + \beta_4 time_{ij} + \beta_5 SNP_i$$

$$+\beta_6 SNP_i * time_{ij} + \sum_{l=1}^{10} \alpha_{il} PC_{il} + U_{0j} + U_{1j} time_{ij} + \varepsilon_{ij},$$

$$\begin{pmatrix} U_{0j} \\ U_{1j} \end{pmatrix} \sim N\left(\begin{pmatrix} 0 \\ 0 \end{pmatrix}, \begin{pmatrix} {\sigma_1}^2 & \rho\sigma_1\sigma_2 \\ \rho\sigma_1\sigma_2 & {\sigma_2}^2 \end{pmatrix} \right), \varepsilon_{ij} \sim N(0, \sigma^2).$$

Once the LMMs were applied to both the KARE and GENIE cohorts, their P-values were combined with inverse variance-weighted meta-analyses using METAL software [59]. The threshold for statistical significance in this model was $P < 5 \times 10^{-08}$, which is conventionally considered to reflect genome-wide significance. To verify the result of LMM, an additional analysis would be needed. Therefore, SNP heritabilities of the average and annual increment of FPG were estimated with GCTA v1.91.7 [60]. The SNP heritability value reflects the relative proportion of phenotypic variance explained by all observed common SNPs. To estimate SNP heritabilities of FPG, we first fit the linear model for repeatedly observed FPGs of each subject as follows:

$$FG_{ij} = \beta_{i0} + \beta_{i1}\left(Age_{ij} - \bar{Age}_{i.}\right) + \varepsilon_{ij}, \varepsilon_{ij} \sim N(0, \sigma^2),$$

where $\bar{Age}_{i.}$ indicates the average of the *i*-th subject's age at different time points, and β_{i0} and β_{i1} indicate the expected FPG when $Age_{ij} = \bar{Age}_{i.}$ and the average annual increment, respectively. $\hat{\beta}_{i0}$ and $\hat{\beta}_{i1}$ were estimated for all subjects in both cohorts, and were analyzed with GCTA. $\hat{\beta}_{i0}$ and $\hat{\beta}_{i1}$ were used as the outcome variable, and $\bar{Age}_{i.}$ and sex were used as covariates.

Table 1.1. Linear mixed model selection by AIC

No.	Assumption for random effect model of LMM	AIC
1	Random intercept	239103.6
2	Uncorrelated random intercept and slope	237120.6
3	Correlated random intercept and slope	237046.9
4	Correlated random intercept and slope with weight*	236851.3

*weight =1/variance of measurement group

A gene-based association method, PrediXcan

To extract the biological meaning from the GWAS results and understand the complex phenomena of various biological pathways, we used a gene-based association method called PrediXcan [61]. To implement this, first we impute the gene expression levels using SNPs on chromosome 10 where significant SNP (rs11187850) was found with the genotype-tissue expression (GTEx) Project data sets [62] as reference transcriptome. Therefore, a total of available 1,041 and 1,076 genes from GTEx data were predicted in KARE and GENIE cohort, respectively. We then correlated these predicted gene expression levels with longitudinal change of FPG defined as the slope of the line by multiple measurements using linear regression model. Regarding the multiple-testing correction approach, we have used Bonferroni correction by the total number of genes tested.

Table 1.2. Summary of samples and SNPs genotyped

Study population	Total	Case (%)	Control (%)	Male (%)	Age (years)	BMI (kg/m^2)	Fasting glucose (mg/dL)	Mean of Follow-up duration (times)	Genotyping platform	SNPs		
										Genotyped†	Imputed‡	Meta
KoGES	6,122	790 (13)	5,332 (87)	2,847 (46)	51.5±8.7	24.4±3.0	84.1±8.5	11 years (5.5 times)	Affymetrix SNP Array 5.0	399,013	3,758,649	2,713,317
GENIE	4,406	237 (5)	4,169 (95)	2,604 (59)	45.7±8.6	23.1±2.9	93.9±9.9	6 years (5.7 times)	Affymetrix KOR_v1.0	344,632	3,692,736	

†Number of genotyped SNPs after quality control (QC): Missingness per SNP < 95%, minor allele frequency (MAF) < 0.05, Hardy-Weinberg equilibrium (HWE) < 1.00×10^{-06}, and sex inconsistency

†Number of imputed SNPs after quality control (QC): MAF < 0.05, HWE <1.00×10^{-06}, imputation quality scores < 0.4

Table 1.3. Annotation for the 2,713,317 variants detected by ANNOVAR

	# of SNPs		# of SNPs
Downstream	15,449	ncRNA_splicing	48
Exonic	16,069	Splicing	47
Exonic;splicing	5	upstream	13,871
Intergenic	1,506,641	Upstream;downstream	418
Intronic	983,956	UTR3	18,417
ncRNA_exonic	7,619	UTR5	3,833
ncRNA_exonic;splicing	3	UTR5;UTR3	8
NcRNA_intronic	146,933		

1.3. Results

Clinical characteristics of study population

A total of 6,122 subjects in the KoGES cohort were investigated at regular intervals of every 2 years, whereas the 4,406 individuals in the GENIE cohort attended voluntary annual health screening. The baseline characteristics of each cohort are described in Table 1.2. During the follow-up period, approximately 10% and 5% of participants had diabetes in the KoGES and GENIE cohort, respectively. The mean age of the KoGES cohort at baseline was 5.8 years older than that of the GENIE cohort and there were more males in KoGES compared to GENIE. The mean fasting glucose of the KoGES cohort at baseline was about 9.8 mg/dL lower than that of the GENIE cohort and the distribution of BMI was similar between the two cohorts. The average increase of FPG per year was 0.7 mg/dL in both cohorts. Information on genotyping platform and number of variants analyzed are presented in Table 1.2. Table 1.3 shows the number of variants in each annotation group categorized by ANNOVAR [56]. Intergenic SNPs and intronic SNPs accounted for 55% and 36% of the total, respectively, with 6% contributed by exonic non-coding RNA.

Genetic variants associated with repeated measures of fasting glucose

First, we investigated genetic variants associated with repeated measures of fasting glucose using LMM as it allows to test both main effect of SNP and SNP*time interaction effect with longitudinal data. A total of four genetic variants were associated with repeated measure of fasting glucose levels with genome-wide significance ($P < 5.0\times10^{-08}$) using the LMM. These include rs12053049 (P =

2.97×10^{-16}) near *G6PC2 (glucose-6-phosphatase catalytic subunit 2)*, rs895636 (P = 2.00×10^{-08}) near *SIX3 (SIX homeobox 3)*, rs2971670 (P = 8.34×10^{-20}) in *GCK* (*glucokinase*), and rs12222793 (P = 4.30×10^{-10}) near *MTNR1B (melatonin receptor 1B)* (Table 2.4). All of these variants have been reported previously to be strongly associated with fasting glucose level [8, 63-66]. Compared to linear model (LM) analysis, LMM was more powerful in identifying genetic loci associated with fasting glucose (Table 1.4). This is evidenced by the fact that all four variants had lower P values in LMM compared to LM. Quantile-quantile (QQ) plot and Manhattan plot for the genetic association using LMM of repeated measures of fasting glucose revealed no evidence for inflation of the test statistics (Figures 1.1 and 1.2). The regional association plots theses genetic loci are shown in Figure 1.3.

Genetic variants associated with longitudinal change in fasting glucose

We further investigated genetic variants associated with longitudinal change in fasting glucose using LMM *SNP*time* interaction term. The statistical model that was used had good control of type 1 error as evidenced by QQ plot and Manhattan plot for genome-wide association of longitudinal change in fasting glucose is shown in Figure 2.1. One variant, rs11187850 in intron of *PLCE1 (phospholipase C epsilon 1)*, achieved genome-wide significant association for longitudinal change in fasting glucose (P = 4.85×10^{-08}). The *PLCE1* gene encodes a phospholipid enzyme that is involved in the hydrolysis of phosphatidylinositol-4,5-bisphosphnate to generate inositol 1,4,5-triphosphate and diacylglycerol. However, its role in diabetes or glucose homeostasis is largely unknown. Three variants had suggestive evidence for an association (P < 1.0×10^{-05}): rs10947494 (P = 3.64×10^{-06})

near *NUDT3 (nucleoside diphosphate linked moiety X-type motif 3)*, rs2414772 ($P = 6.30\times10^{-06}$) near *MIR6085 (MicroRNA 6085)*, and rs16959641 ($P = 2.64\times10^{-06}$) near *USB1 (U6 snRNA biogenesis phosphodiesterase 1*) (Table 2.5). Regional association plots of these four variants are shown in Figure 3. We fitted a simple linear model with fasting glucose as the outcome variable and follow-up time as the independent variable, categorized by the three genotypes of the rs11187850 variant which showed genome-wide significant association. The rs11187850 G was significantly associated with increased slope of fasting glucose over time in both cohorts. The reason for the difficulty in identifying the SNPs affecting the change in FPG over time can be illuminated based on the GCTA analysis results. As shown in Table 2.6, the GCTA estimated a 10% marginal influence of the SNP effect, whereas the influence of the SNP-by-time interaction was only 4%. Based on these results, genetic effects on longitudinal change of fasting glucose are likely small.

Next, we compared the results to those of previous GWAS results for fasting glucose change from European participants [11]. In this study, the inverse normal transformed slopes in the longitudinal cohort were used as the outcome variable (i.e, fasting glucose change) for the analysis. Then, they conducted inverse variance weighted meta-analysis using 13,807 non-diabetic individuals of European descent from nine cohorts. They showed six suggestive associated variants with *P*-value $< 5 \times 10^{-06}$; rs7114256 ($P = 8.78\times10^{-07}$) near *ODZ4 (Protein Odd Oz/ten-m homolog 4)*, rs606243 ($P = 1.42\times10^{-06}$) near *ALLC (Allantoicase)*, rs17496593 ($P = 2.12\times10^{-06}$) near *NUDT12 (Nudix Hydrolase 12)*, rs17496653 ($P = 2.58 \times 10^{-06}$) near *NUDT12*, rs17562893 ($P = 3.78 \times 10^{-06}$) near *NUDT12* and rs7103693 ($P = 4.19 \times 10^{-06}$) near *ODZ4*. However, we failed to replicate these variants in the present

research. Furthermore, the genome wide significant SNP, rs11187850 from our study (P=4.85×10-08) is largely different to those that have been discovered using European GWAS (P=0.769). Therefore, we conducted meta-analysis using summary results of European study with our results using Fisher's method and inverse variance weighted analysis. Because of the different genotyping quality control criteria for MAF in European studies (MAF>0.01) and ours (MAF>0.05), a total of 1,019,144 overlapping SNPs was used for meta-analysis. We found a several suggestive signal ($P < 5.00\times10^{-06}$) for glucose change over time, but the results differed depending on which method of meta-analysis was applied. Top 10 suggestive SNPs associated with longitudinal change of FPG identified by both methods were respectively presented in supplement Table 1.10.

Association of known glycemic variants with longitudinal change in fasting glucose

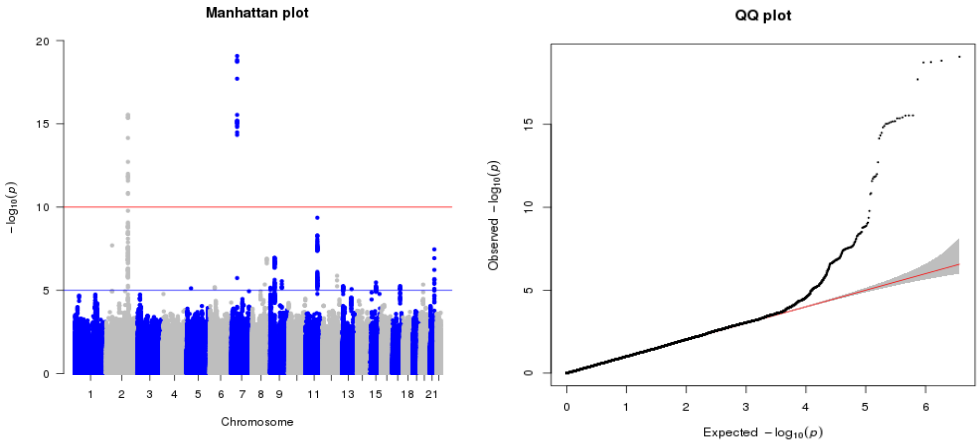
We investigated whether rs11187850 in *PLCE1* was associated with risk of type 2 diabetes and increased fasting glucose. To visualize the longitudinal change trend for different SNP groups (the number of copies of minor allele is 0, 1, or 2), the time was plotted on the x-axis, and the fasting glucose was on the y-axis, and the plot was drawn by SNP groups (Figure 1.4). This variant did not show association with increased risk of type 2 diabetes ($P = 2.91\times10^{-06}$) nor increased fasting glucose level ($P = 4.75\times10^{-04}$) in LMM. A limited number of known genetic variants of type 2 diabetes, and fasting glucose were nominally associated with longitudinal change in fasting glucose. Still none of the variants were significant when multiple comparison was considered. These findings suggest that the variants associated with

fasting glucose do not have significant impact on longitudinal change in glucose level over time (Table 1.8 and 1.9).

Gene expression levels of target gene (*NOC3L* and *PLCE1*) associated with artery, heart and esophagus tissue

We applied PrediXcan to test the molecular mechanisms through which genetic variation affects longitudinal change of FPG. A total of genotyped and imputed SNPs on chromosome 10 were used to predict gene expression levels using the weights derived from reference transcriptome data sets with GTEx. The outcome variable defined as the slope of the line by multiple measurements of FPG using linear regression model and the age, sex, and BMI were used as covariates. We then regress the predicted imputed gene expression levels on the phenotype of interest using linear regression model in each of the forty-eight tissue with target genes (*PLCE1, PLCE1-AS1, PLCE1-AS2, NOC3L,* and *TBC1D12*). In the results of KARE, expression levels of *NOC3L* gene associated with Artery ($P = 0.048$) and Heart tissue ($P = 0.044$), but this result are not replicated in GENIE cohort suggesting that expression levels of *PLCE1* was associated esophagus ($P = 0.012$) (Table 1.11).

(A)



(B)

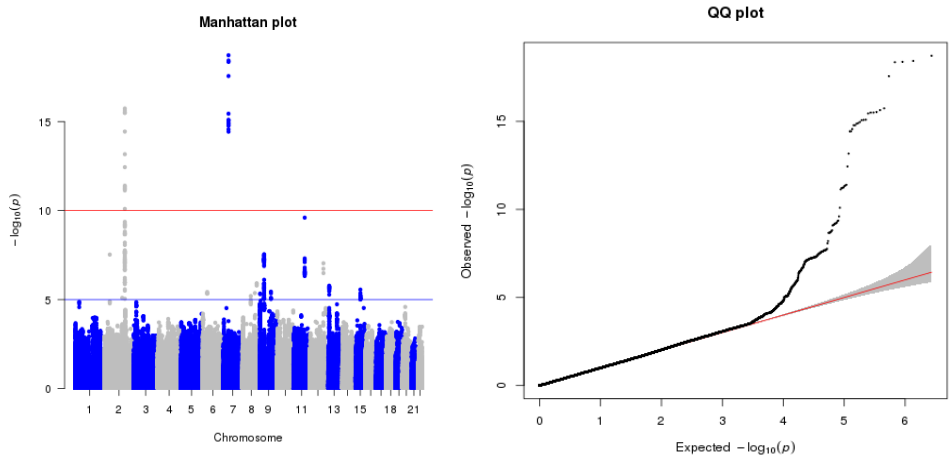
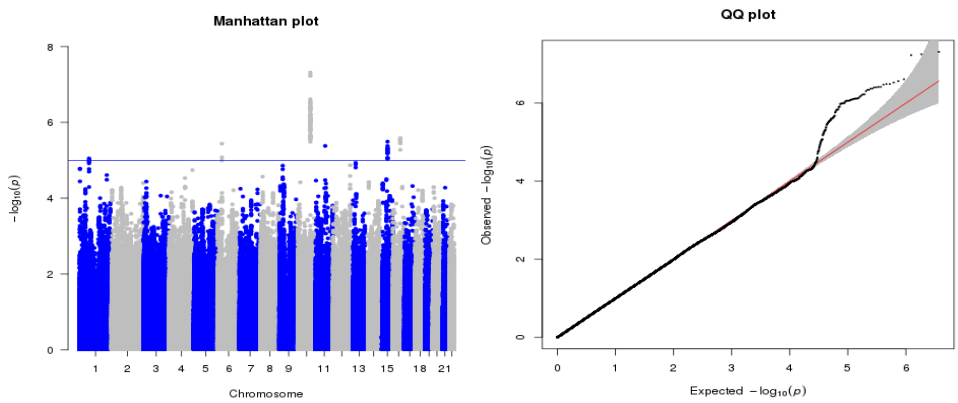


Figure 1.1. Manhattan and quantile-quantile plot for SNP effects
(A) BMI-adjusted (B) BMI-unadjusted

(A)



(B)

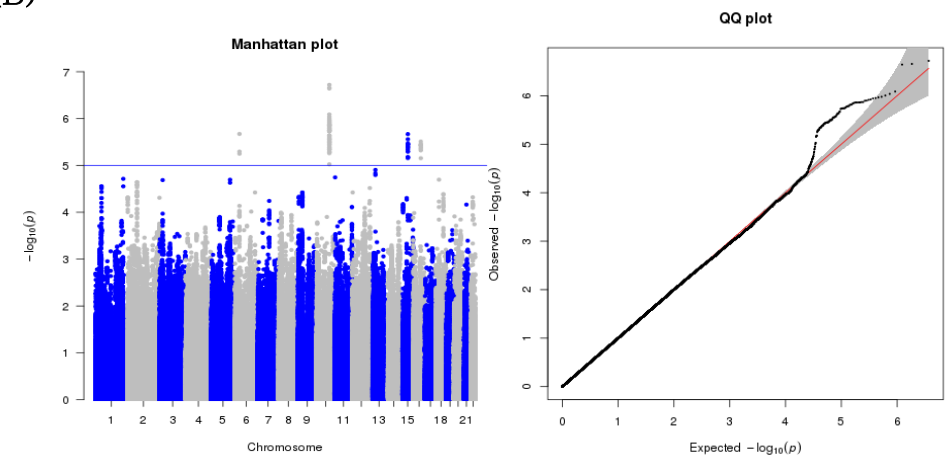


Figure 1.2. Manhattan and quantile-quantile plot for SNP*time effects
(A) BMI-adjusted (B) BMI-unadjusted

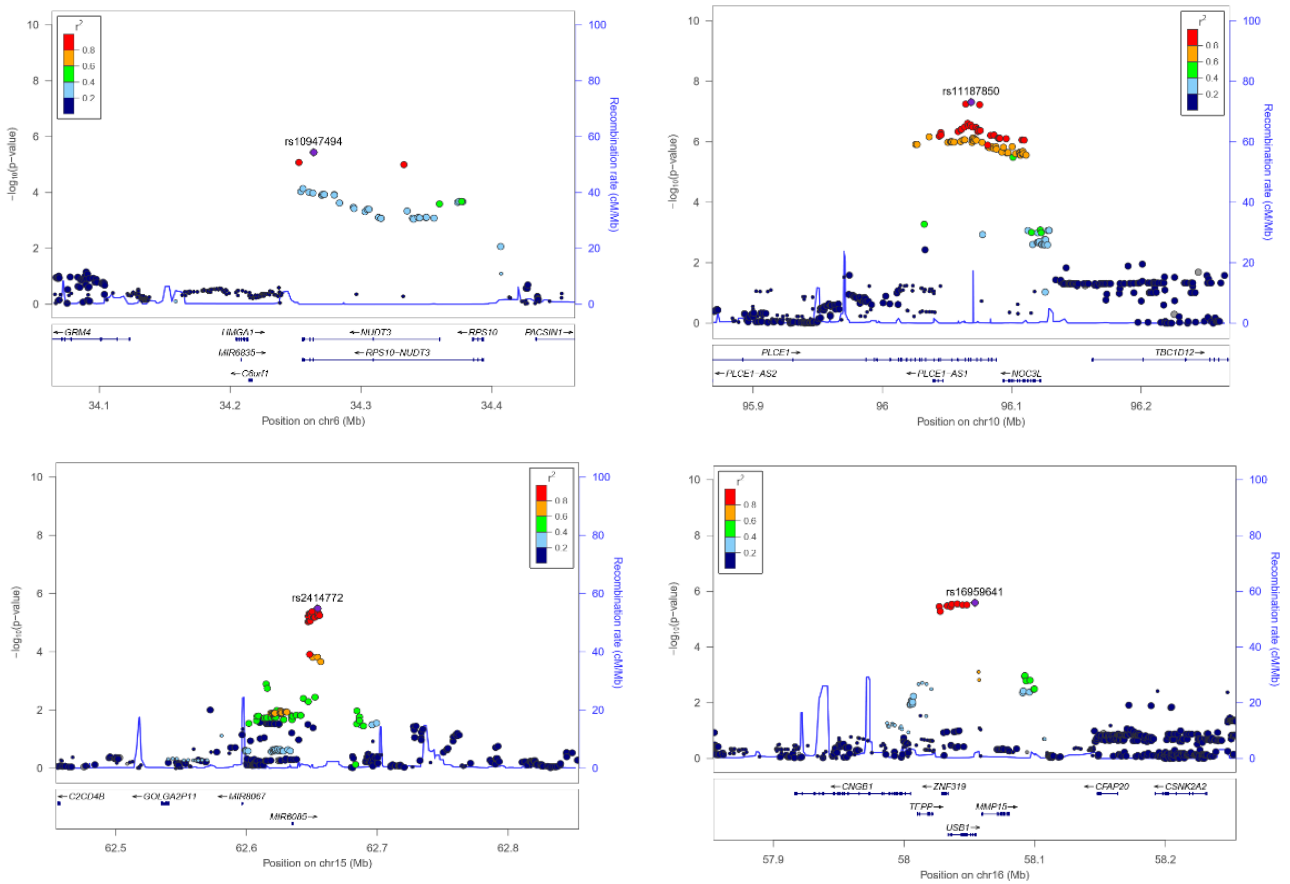


Figure 1.3 LocusZoom of SNP*time association in meta-analysis (P <1.0×10^{-06})

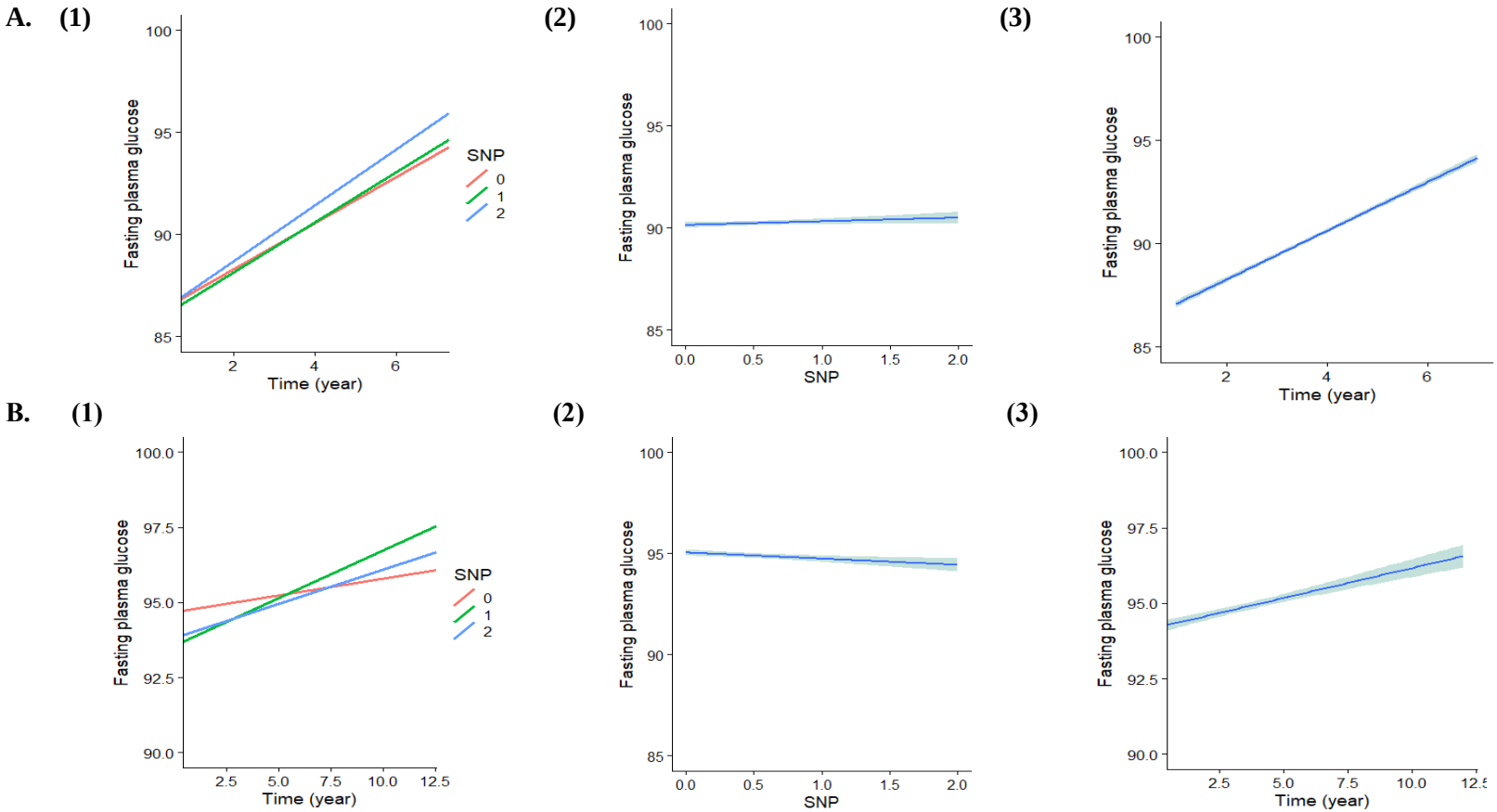


Figure 1.4. SNP and age interaction and main Plot **A.** KARE **B.** GENIE

Table 1.4. Power comparison between linear mixed model (LMM) and linear model (LM)

Cohort	Chr	SNP	Locus	*P* (LMM)	*P* (LM)	ANNOVAR
KARE	2	rs12053049	169767148	7.11×10^{-11}	9.16×10^{-11}	*G6PC2* (downstream)
	7	rs1799884	44229068	5.75×10^{-13}	2.05×10^{-08}	*GCK* (upstream)
	11	rs12222793	92667047	1.15×10^{-08}	4.71×10^{-06}	*FAT3*(dist=37412), *MTNR1B*(dist=35742) (intergenic)
GENIE	7	rs2971670	44226101	2.18×10^{-08}	8.54×10^{-06}	*GCK* (intronic)
	21	rs2877119	47308530	2.67×10^{-09}	7.39×10^{-07}	*PCBP3* (intronic)

Table 1.5. Genome-wide significant variants with evidence of SNP association in meta-analysis ($P < 5.0 \times 10^{-08}$)

Chr	SNP	Locus	A*	Independent study						META		ANNOVAR
					Total	Effect	SE	AF†	*P*‡	Type (info)	N (direction)	*P* (HetPVal)	
2	rs12053049	169767148	T/C	KARE	6,122	1.008	0.154	0.354	7.10×10^{-11}	imputed (0.981)	10,528 (++)	2.97×10^{-16} (0.6595)	*G6PC2* (downstream)
				GENIE	4,406	1.036	0.209	0.341	7.34×10^{-07}	imputed (0.972)			
2	rs895636	45188353	C/T	KARE	6,122	0.574	0.151	0.378	1.43×10^{-04}	imputed (0.882)	10,528 (++)	2.00×10^{-08} (0.4608)	*SIX3*(dist=15137),*SIX2*(dist =43971) (intergenic)
				GENIE	4,406	0.831	0.198	0.371	2.75×10^{-05}	genotyped (1.000)			
7	rs2971670	44226101	C/T	KARE	6,122	1.346	0.187	0.188	6.17×10^{-13}	imputed (0.996)	10,528 (++)	8.34×10^{-20} (0.6983)	*GCK* (intronic)
				GENIE	4,406	1.400	0.250	0.182	2.18×10^{-08}	imputed (0.999)			
11	rs12222793	92667047	A/G	KARE	6,122	0.827	0.144	0.514	1.14×10^{-08}	genotyped (1.000)	10,528 (++)	4.30×10^{-10} (0.1428)	*FAT3*(dist=37412),*MTNR1B* (dist=35742) (intergenic)
				GENIE	4,406	0.561	0.192	0.516	3.49×10^{-03}	genotyped (1.000)			

* reference/alternative allele, † alternative allele frequency, ‡ P-value form linear mixed model (LMM)

Table 1.6. Top four variants with suggestive evidence of SNP*time association in meta-analysis ($P < 1.0 \times 10^{-05}$)

Chr	SNP	Locus	A	Independent study						META		ANNOVAR
					Total	Effect	SE	AF	*P*	Type (info)	N (direction)	*P* (HetPVal)	
6	rs10947494	34263743	A/G	KARE	6,122	0.213	0.046	0.205	4.53×10^{-06}	imputed (0.992)	10,528 (++)	3.64×10^{-06} (0.1032)	*NUDT3,RPS10-NUDT3* (intronic)
				GENIE	4,406	0.120	0.068	0.200	7.96×10^{-02}	genotyped (1.000)			
10	rs11187850	96068480	A/G	KARE	6,122	0.152	0.042	0.255	3.49×10^{-04}	imputed (0.996)	10,528 (++)	4.85×10^{-08} (0.3659)	*PLCE1* (intronic)
				GENIE	4,406	0.266	0.063	0.250	2.45×10^{-05}	imputed (0.996)			
15	rs2414772	62654213	G/A	KARE	6,122	-0.163	0.039	0.681	3.26×10^{-05}	imputed (0.998)	10,528 (--)	6.30×10^{-06} (0.2719)	*MIR6085*(dist=18876), *MGC15885* (dist=275158) (intergenic)
				GENIE	4,406	-0.134	0.058	0.671	2.14×10^{-02}	imputed (0.985)			
16	rs16959641	58054099	C/G	KARE	6,122	0.222	0.068	0.078	1.09×10^{-03}	imputed (0.954)	10,528 (++)	2.46×10^{-06} (0.6144)	*USB1* (exonic)
				GENIE	4,406	0.366	0.107	0.076	6.00×10^{-04}	genotyped (1.000)			

** A=reference/alternative allele, AF=alternative allele frequency

Table 1.7. BMI adjusted and unadjusted results for top four variants

Chr	SNP	Locus	A	BMI adjusted			BMI unadjusted		
				Beta	SE	*P* (HetPVal)	Beta	SE	*P* (HetPVal)
6	rs10947494	34263743	A/G	0.184	0.039	3.64×10^{-06} (0.103)	0.181	0.038	2.13×10^{-06} (0.277)
10	rs11187850	96068480	A/G	0.187	0.035	4.85×10^{-08} (0.365)	0.182	0.035	1.90×10^{-07} (0.087)
15	rs2414772	62654213	G/A	-0.154	0.033	6.30×10^{-06} (0.271)	-0.153	0.032	2.15×10^{-06} (0.775)
16	rs16959641	58054099	C/G	0.261	0.052	2.46×10^{-06} (0.614)	0.265	0.056	3.09×10^{-06} (0.345)

Table 1.8. Variants with suggestive evidence of SNP*time association nominally ($P < 0.05$) associated with longitudinal change in fasting glucose among the known loci for T2D

Chr	SNP	Locus	A	Independent study						META		ANNOVAR
					Total	Effect	SE	AF	*P*	Type (info)	N (direction)	*P* (HetPVal)	
1	rs2296172	39835817	A/G	KARE	6,122	0.145	0.051	0.143	5.08×10^{-03}	genotyped (1.000)	10,528 (++)	3.42×10^{-03} (0.3787)	*MACF1* (exonic)
				GENIE	4,406	0.097	0.079	0.144	2.21×10^{-01}	genotyped (1.000)			
2	rs243021	60584819	G/A	KARE	6,122	0.073	0.038	0.668	5.81×10^{-02}	imputed (0.986)	10,528 (++)	3.37×10^{-02} (0.6702)	*LOC101927285*(dist =1078284),*MIR443 2HG*(dist=1532) (intergenic)
				GENIE	4,406	0.060	0.057	0.666	2.94×10^{-01}	genotyped (1.000)			
7	rs864745	28180556	T/C	KARE	6,122	0.070	0.040	0.271	8.51×10^{-02}	genotyped (1.000)	10,528 (++)	4.90×10^{-02} (0.7329)	*JAZF1* (intronic)
				GENIE	4,406	0.062	0.061	0.273	3.11×10^{-01}	genotyped (1.000)			
9	rs10965250	22133284	G/A	KARE	6,122	-0.094	0.036	0.424	9.85×10^{-03}	imputed (0.985)	10,528 (--)	2.93×10^{-03} (0.6288)	*CDKN2B-AS1*(dist=12191),*D MRTA1*(dist=313556) (intergenic)
				GENIE	4,406	-0.086	0.055	0.449	1.19×10^{-01}	genotyped (1.000)			
13	rs9552911	23864657	G/A	KARE	6,122	-0.084	0.044	0.221	5.66×10^{-02}	genotyped (1.000)	10,528 (--)	4.89×10^{-02} (0.5321)	*SGCG* (intronic)
				GENIE	4,406	-0.053	0.066	0.229	4.25×10^{-01}	imputed (0.970)			

** A=reference/alternative allele, AF=alternative allele frequency

Table 1.9. Variants with suggestive evidence of SNP*time nominally ($P < 0.05$) associated with longitudinal change in fasting glucose among the known loci for fasting glucose

Chr	SNP	Locus	A	Independent study						META		ANNOVAR
					Total	Effect	SE	AF	*P*	Type (info)	N (direction)	*P* (HetPVal)	
7	rs6943153	50791579	T/C	KARE	6,122	-0.056	0.041	0.739	1.80×10^{-01}	imputed (0.996)	10,528 (--)	3.44×10^{-02} (0.6715)	*GRB10* (intronic)
				GENIE	4,406	-0.105	0.062	0.734	9.07×10^{-02}	imputed (0.980)			
9	rs10811661	22134094	T/C	KARE	6,122	-0.090	0.036	0.426	1.23×10^{-02}	genotyped (1.000)	10,528 (--)	5.10×10^{-03} (0.5725)	*CDKN2B-AS1*(dist=13001),*DMRTA1* (dist=312746) (intergenic)
				GENIE	4,406	-0.076	0.055	0.450	1.67×10^{-01}	genotyped (1.000)			
13	rs2293941	28491198	G/A	KARE	6,122	0.084	0.037	0.469	2.41×10^{-02}	imputed (0.982)	10,528 (++)	2.90×10^{-02} (0.3615)	*PDX1-AS1* (ncRNA_intronic)
				GENIE	4,406	0.039	0.055	0.462	4.73×10^{-01}	genotyped (1.000)			

** A=reference/alternative allele, AF=alternative allele frequency

Table 1.10. Results of GCTA

	β_{i0} (intercept)	β_{i1} (slope)
genetic variance (standard error, SE)	5.34 (1.97)	0.24 (0.17)
residual variance (SE)	50.94 (2.06)	4.89 (0.18)
phenotypic variance (SE)	56.29 (0.77)	5.14 (0.07)
ratio of genetic variance to phenotypic variance (SE)	0.10 (0.03)	0.04 (0.03)
P-VALUE	3.52×10^{-03}	7.58×10^{-02}

Table 1.11. Top 10 variants associated with longitudinal change in FPG from meta-analysis using Fisher and METAL method

Chr	SNP	A*	Korean					European					Fisher's *P*
			BP	N	Beta	SE	*P*	BP	N	Beta	SE	*P*	
1	rs17267561	A/G	211540941	10528	0.290	0.067	2.45×10^{-05}	209607564	13001	-0.033	0.019	0.076	2.65×10^{-05}
2	rs4852715	A/T	71088070	10528	0.044	0.043	0.348	70941578	13005	0.094	0.021	6.79×10^{-06}	3.29×10^{-05}
4	rs7671458	T/G	142627461	10528	-0.109	0.067	0.123	142846911	13006	0.079	0.018	2.13×10^{-05}	3.61×10^{-05}
6	rs10947494	A/G	34263743	10528	0.184	0.039	3.65×10^{-06}	34371721	13756	-0.035	0.013	8.69×10^{-03}	5.78×10^{-07}
6	rs9376062	T/C	135057244	10528	-0.061	0.031	0.046	135098937	12992	-0.058	0.013	8.98×10^{-06}	6.48×10^{-06}
8	rs2614079	A/G	28168659	10528	-0.133	0.044	2.48×10^{-03}	28224578	12977	-0.091	0.022	2.98×10^{-05}	1.29×10^{-06}
10	rs11187850	A/G	96068480	10528	0.187	0.035	4.86×10^{-08}	96058470	13004	0.0042	0.014	0.769	6.77×10^{-07}
12	rs11061537	T/C	131929543	10528	-0.118	0.033	3.63×10^{-04}	130495496	12994	0.038	0.014	5.80×10^{-03}	2.96×10^{-05}
15	rs2414772	A/G	62654213	10528	-0.154	0.033	3.22×10^{-06}	60441505	13006	-0.021	0.015	0.148	7.40×10^{-06}
16	rs3743559	A/C	58035429	10528	0.259	0.057	3.59×10^{-06}	56592930	12995	0.032	0.024	0.182	9.97×10^{-06}
Chr	SNP	A*	Korean					European					METAL *P*
			BP	N	Beta	SE	*P*	BP	N	Beta	SE	*P*	
2	rs4852230	C/T	71084562	10528	0.049	0.043	0.289	70938070	12996	0.093	0.021	1.39×10^{-05}	7.57×10^{-06}
2	rs630313	C/T	4498784	10528	-0.035	0.031	0.275	4476659	13006	-0.065	0.015	2.13×10^{-05}	1.15×10^{-05}
3	rs13064000	G/A	2440071	10528	0.097	0.066	0.095	2415071	12944	0.062	0.015	5.10×10^{-05}	1.33×10^{-05}
4	rs6842735	G/T	142639407	10528	-0.110	0.067	0.118	142858857	13006	-0.076	0.018	3.88×10^{-05}	6.64×10^{-06}
4	rs17287504	C/T	159863382	10528	-0.107	0.059	0.065	160082832	12978	-0.054	0.014	1.86×10^{-04}	3.03×10^{-05}
5	rs17496653	G/A	104227288	10528	0.013	0.060	0.801	104255187	13005	0.110	0.023	2.75×10^{-06}	5.48×10^{-06}
6	rs9376062	T/C	135057244	10528	-0.061	0.030	0.046	135098937	12992	-0.058	0.013	8.98×10^{-06}	1.09×10^{-06}
6	rs9392166	A/G	7643407	10528	-0.083	0.031	0.011	7588406	12906	-0.054	0.016	8.87×10^{-04}	2.46×10^{-05}
8	rs2614079	A/G	28168659	10528	-0.132	0.044	2.48×10^{-03}	28224578	12977	-0.091	0.022	2.98×10^{-05}	4.77×10^{-07}
13	rs1572067	T/G	24092276	10528	-0.109	0.040	8.80×10^{-03}	22990276	12238	-0.051	0.015	1.01×10^{-03}	3.46×10^{-05}

* reference/alternative allele

Table 1.12. Significant PrediXcan results for the KARE using target genes

Study	Gene symbol	Description	Beta	*Se*	*P*	Tissue
KARE	*NOC3L*	NOC3 Like DNA Replication Regulator	1.056	0.534	0.048	Artery
	NOC3L		1.216	0.602	0.044	Heart
GENIE	*PLCE1*	phospholipase C epsilon 1	0.893	0.356	0.012	Esophagus

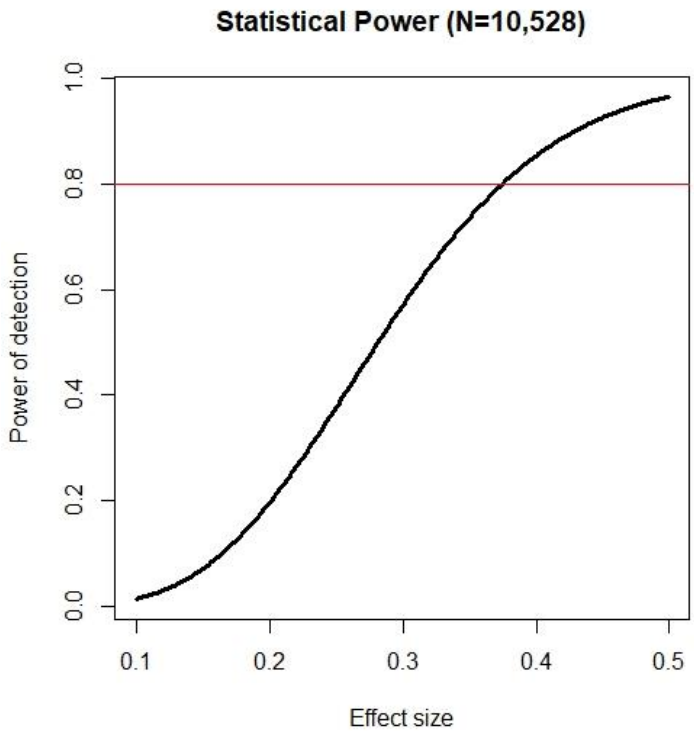


Figure 1.5 Power analysis

1.4. Discussion

Our study provides the first comprehensive assessment of genetic variants of fasting glucose change over time in the Korean population cohort with repeated fasting glucose measures. To date, in Korea, little of genetic risk factors for longitudinal deterioration of fasting glucose has been explained and not been widely studied. The most important finding of this study was that only a variant in *PLCE1* achieved a genome-wide significance level ($P < 5.0\times10^{-08}$) and suggest variants with suggestive evidence of *SNP*time* association nominally ($P < 0.05$) associated with longitudinal change in fasting glucose among the known loci for fasting glucose and T2D.

Our findings are in line with results from recent research in meta-analysis with nine cohorts of European representing three continents (America, Europe and Australia), in which there are no genome-wide significant association with fasting glucose change over time. This study was a large-scale study conducted with a total of 13,807 participants. Statistical power of 80% with a type-1 error rate of 5.0×10^{-08} achieved to detect a genetic variant having at least 0.28 and 0.37 effect size in European and Korean studies, respectively but none of the genome-wide significant SNPs were obtained. Therefore, we expected that the meta-analysis with European summary results would be improve the power to explore the genetic variants affecting the longitudinal change of fasting glucose, but no meaningful results were obtained. The SNP that genome-wide significantly in our study was not significant at all in European studies, and the genetic variations suggested in the European studies were not replicated in our study. There may be several reasons for the lack of significant signals. Firstly, just as the genetic variation affecting FPG due

to race differences is slightly different in Asian and the European, the genetic mechanisms affecting FPG longitudinal changes may be different. In addition to genetic variation, changes in FPG may have resulted in differences in outcomes due to a combination of environmental effects such as eating habits in the Asian and the European. Technically, we used mixed model for each repeated measure but in European study the longitudinal patterns of fasting glucose were summarized into an individual slope as an outcome in linear model. These reduced data (i.e, slopes) were inverse normal transformed which made interpretation of the research results difficult. Additionally, unlike our research, BMI was not considered in the statistical model in European studies. BMI has been reported to increase steadily until just before diagnosis of diabetes, which means BMI is an important factor for longitudinal change of fasting glucose. Therefore, missing important covariates may have made a big difference in the study results. Finally, different time points and follow-up time between cohorts used in both meta-analyses are treated as factors to drive the heterogeneity issue in meta-analysis.

In conclusion, longitudinal cohorts with large sample size, the prospective design, and longterm follow-up periods in the Korean population allowed us to investigate loci influencing fasting glucose change over time. Our results show that only a novel variant achieved statistical genome-wide significance threshold, but the mechanism by which variation in *PLCE1* cannot fully be accounted for biologically. Further replication studies using longitudinal cohorts with larger sample size and homogeneous populations (e.g. consistent of longitudinal measurement interval and time) are required to validate our findings. If the analysis proceeds, considering

repeat measurements in multiple time points would be increase statistical power compared to a single or average measurement.

PART II. Causal Evaluation of Laboratory Markers in Type 2 Diabetes on Cancer and Vascular Diseases Using Various Mendelian Randomization Tools

2.1. Research Problem

Type 2 diabetes (T2D) is characterized by high blood sugar, insulin resistance, and a relative lack of insulin and represents a common metabolic disorder worldwide. In its early stage, T2D is easy to ignore due to the lack of symptoms; however, chronic or poorly controlled T2D leads to eventually disabling or life-threatening complications. Numerous epidemiological studies have consistently demonstrated increased risks of cancer, vascular disease, nerve damage, and poor health-related outcomes in T2D patients [67-69], resulting in a shorter life expectancy [21]. The main T2D-related complications reported in large-scale epidemiological studies tend to be malignant solid tumors [70] and cardiovascular disease, including ischemic heart disease and stroke [71-74]. However, the causal relationship between T2D and diverse health-related outcomes needs to be investigated and compared.

Fasting plasma glucose (FPG) levels ≥126 mg/dL or post-challenge 2-h postload glucose (2h-PG) levels ≥200 mg/dL in a 75-g 2-h oral glucose tolerance test (2h-OGTT) have been used as diagnostic criteria for T2D. Additionally, hemoglobin A1c (HbA1c) levels ≥6.5% were added to these diagnostic criteria in 2010 [75, 76]. The three tests (FPG, 2h-PG, and HbA1c) are dependent on blood glucose metabolism status. Specifically, FPG assesses the state of stable sugar levels in the body following a temporary increase in externally administered sugar. The 2h-

OGTT indicates how efficiently insulin is processed during metabolism in response to increased externally administered glucose. HbA1c reflects the average blood sugar level until immediately before the test and not at the time of sample collection, because hemoglobin increases with time and according to glucose concentration [77, 78]. Therefore, it is necessary to investigate the causal effects of these three T2D-related traits in the blood and how they differ in subsequent pathological disorders.

To efficiently identify causal associations between T2D-related traits and various phenotypes without potential biases or confounding and/or reverse causations, Mendelian randomization (MR) can be used to assess how genetic variants act as instruments for instrumental variable (IV) analysis aimed at estimating the causal effect of one trait on another. Using genetic variants as instruments, which are not associated with conventional confounders of observational studies, allows the MR approach to be considered analogous to randomized controlled trials [27]. MR analysis requires three assumptions: 1) IVs are strongly associated with intermediate exposure, 2) IVs are independent of confounders, and 3) IVs affect outcomes only through the exposure path. If these assumptions hold, an inverse-variance-weighted (IVW) method provides the most efficient and unbiased estimates of causal effects [79]. Various MR methods have been proposed for providing a more robust approach under weaker assumptions [23-26, 28].

The aim of this study was to assess the causal effect of T2D-related traits (FPG, 2h-PG, and HbA1c) on cancers and vascular diseases via MR analysis using several methods, including those measuring sensitivity in the MR-Base platform database [80].

2.2. Materials and Methods

Exposure datasets

The exposure traits of interest were FPG, 2h-PG, and HbA1c. The summary statistics for T2D-related traits were obtained through large-scale genome-wide association study (GWAS) meta-analyses of 133,010 non-diabetic individuals from collaborating studies within the Meta-Analysis of Glucose and Insulin related traits Consortium (MAGIC) [10]. In most of these studies, participants were of European ancestry and adults. A total of ~2.5 million genome-wide directly genotyped or imputed autosomal single-nucleotide polymorphisms (SNPs) were reported, including 36, 9, and 11 SNPs with genome-wide significant ($P < 5 \times 10^{-08}$) associations with FPG, 2h-PG, and HbA1c, explaining 4.8%, 1.7%, and 2.4% of the variance in the trait, respectively. Among these, SNPs were selected as IV candidates not in linkage disequilibrium (LD; r^2<0.001) or within 10,000 kb of an established signal. To specify final IV sets, available genetic instruments for assessing outcome traits of interest were explored via the MR-Base platform database (https://www.mrbase.org/) or through the R package 'TwoSampleMR' (https://rdrr.io/github/MRCIEU/TwoSampleMR/). To reflect the same reference strand between exposure and outcome, alleles and effects were harmonized using effect/non-effect alleles and minor allele frequency for palindromic SNPs.

Outcome datasets

Human phenotypes were divided into two categories of diseases or traits known to be related to T2D. The first category was cancer at major sites: breast, gall bladder, lung [adenocarcinoma and squamous cell (SC) carcinoma], ovarian, pancreatic, and thyroid (differentiated types). The second category was vascular disease: coronary kidney disease (CKD), coronary artery disease (CAD), stroke, cardio-embolic stroke, small-vessel stroke, and high-density lipoprotein (HDL)/low-density lipoprotein (LDL) cholesterol levels. We obtained summary SNP-outcome associations with a total of 14 human health phenotypes through the MR-BASE platform. Additionally, information regarding each outcome trait of interest was extracted (e.g., author/study/consortium name, number of cases and controls, publication year, PubMed ID, study population, unit, etc.) and listed in Table 2.1.

Table 2.1. Description of data from MR-Base according to phenotype.

Category	Trait	Consortium/ First author	PubMed ID	Unit	No. of cases	No. of controls	No. of SNPs	Population
1. Cancer								
	Breast cancer	BCAC	25751625	LogOR	15,748	18,084	13,011,123	European
	Lung cancer	ILCCO	24880342	LogOR	11,348	15,861	8,945,893	European
	Lung cancer (SC)	ILCCO	24880342	LogOR	3,275	150,038	8,893,750	European
	Ovarian cancer	OCAC	28346442	LogOR	1,366	40,941	11,403,952	European
	Pancreatic cancer	PanScan1	19648918	LogOR	1,896	1,939	521,863	European
	Thyroid cancer	Kohler A	23894154	LogOR	649	431	572,028	European
2. Vascular disease								
	CAD	VanderHarst P	29212778	LogOR	122,733	424,528	7,934,254	European
	CKD	CKDGen	26831199	LogOR	12,385	104,780	2,191,877	Mixed
	HDL cholesterol	GLGC	24097068	SD	—	187,167	2,447,442	Mixed
	LDL cholesterol	GLGC	24097068	SD	—	173,082	2,437,752	Mixed
	Stroke	Malik R	29531354	LogOR	40,585	406,111	7,633,440	European
	(Cardio-embolic)	Malik R	29531354	LogOR	7,193	406,111	8,271,294	European
	(Small-vessel)	Malik R	29531354	LogOR	5,386	192,662	6,150,261	European

SC, squamous cell; SD, standard deviation; CKD, chronic kidney disease; CAD, coronary artery disease; LDL, low-density lipoprotein; HDL, high-density lipoprotein; logOR, log odds ratio.

MR assumptions

The assumptions of MR studies can be represented using causal directed acyclic graphs (DAG) (Figure 2.1). In a DAG, the genetic variant G_j (j=1, 2, …, J) and the exposure, X, are denoted as γ_j, and the association between the genetic variant, G_j, and the outcome, Y, is denoted as α_j. Associations between a confounding factor (U) and G_j, X, and Y are denoted as ψ_j, K_x, and K_y, respectively. In a two-sample MR setting, we refer to $\hat{\gamma}_j$ as an estimate from the j^{th} SNP-exposure association (with variance $\sigma^2_{X_j}$) from sample 1 and $\hat{\alpha}_j$ as an estimate from the j^{th} SNP-outcome association (with variance $\sigma^2_{Y_j}$) from sample 2.

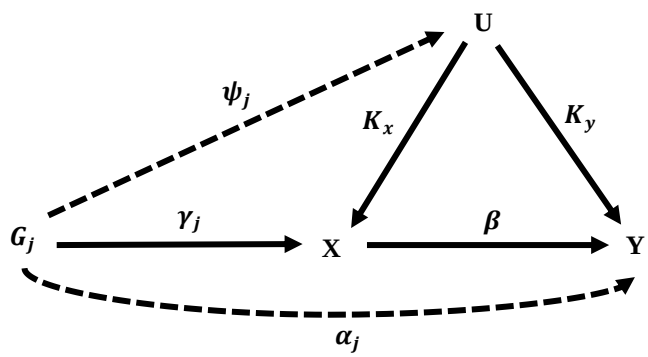


Figure 2.1. Causal directed acyclic graph for MR analysis.

$$\text{Sample 1: } \hat{\gamma}_j = \gamma_j + k_x\varphi_j + \epsilon_{X_j},\ \text{var}\left(\epsilon_{X_j}\right) = \sigma^2_{X_j}$$

$$\text{Sample 2: } \hat{\alpha}_j = \alpha_j + k_y\varphi_j + \beta\left(\gamma_j + k_x\varphi_j\right) + \epsilon_{Y_j},\ \text{var}\left(\epsilon_{Y_j}\right) = \sigma^2_{Y_j}$$

The genetic variant, G_j, for valid IVs must satisfy the following three core assumptions; (i) IV1: $\gamma_j \neq 0$, (ii) IV2: $\varphi_j = 0$, (iii) IV3: $\alpha_j = 0$. Furthermore, MR requires a "NO Measurement Error" (NOME) assumption and an Instrument Strength Independent of Direct Effect (InSIDE) assumption. It is important to assess

the instrument strength to prevent weak instrument bias on MR analysis. We evaluated weak instruments with mean F-statistics, and the degree of violation of the NOME assumption was quantified using the previously reported I^2 statistic (ranging 0–1) [23]. Higher values for I^2 indicate lesser dilution of the causal effect estimate.

MR methods

Using all genetic variants, G_j, that satisfy the three IV assumptions and the NOME and InSIDE assumptions, the causal effect of exposure on the outcome can be consistently estimated from the ratio estimates and averaged using an IVW method [24]. The IVW estimate is the most efficient method when all genetic variants satisfy all three IV assumptions. Cochran's Q statistic was used to quantify heterogeneity [81].

However, the estimate could be biased if one or more variants are invalid. The weighted median method provides valid causal estimates, even if up to 50% of the instruments are invalid. The median is unaffected by outliers, making the weighted median estimate insensitive to a pleiotropic genetic variant. Causal effects are obtained from the weighted median of the ratio estimates in genetic instruments, resulting in smaller standard errors receiving more weight [25].

The MR-Egger method allows all SNPs to be used as invalid instruments but requires variants to satisfy the InSIDE assumption, enabling estimation of appropriate causal effects in the presence of pleiotropic effects [26]. This model is suitable for linear regression and the intercept term, β_{0E}, is interpreted as the average horizontal pleiotropic effect across the genetic variants [26]. Rücker's Q'

statistic from MR-Egger was used to quantify directional heterogeneity [81]. If estimates of β_{0E} equal zero, the MR-Egger slope estimate will be the same as the IVW estimate [27]. However, when the I^2 statistic quantifying the strength of NOME violation for IVs for the MR-Egger method is low, a magnitude of regression dilution still occurs. In cases where the NOME assumption is violated, the SIMEX method can be used to correct attenuation bias [23].

Violation of IV3 (no horizontal pleiotropy) can raise a severe bias in MR analysis. The MR-PRESSO test has an advantage over MR-Egger, in that it identifies and removes pleiotropic SNPs. The test comprises three parts: 1) the MR-PRESSO global test detects horizontal pleiotropy, 2) the outlier-corrected causal estimate corrects for the detected horizontal pleiotropy, and 3) the MR-PRESSO distortion test estimates whether the causal estimates differ significantly ($P < 0.05$) following adjustment for the outliers [28]. Therefore, MR-PRESSO results are preferable in the presence of a horizontal pleiotropic effect.

The appropriate methods differ according to the assumptions satisfied, and the most suitable choices are presented in Table 3.2 and 3.3. The IVW method is the most efficient way to estimate the causal effect when all genetic variants are valid instruments [79]. In cases where the MR assumption of no pleiotropy is not met, the MR-PRESSO test detects possible outliers and provides consistent estimates following outlier removal [82]. When some genetic variants are invalid (<50%), the weighted median approach can be used as an alternative method of providing a consistent estimate [25]. By contrast, MR-Egger can obtain a causal estimate by correcting directional pleiotropy but has the disadvantage of low power [26]. If the NOME assumption is violated (I^2 < 90%), the MR-Egger (SIMEX) method would

be suitable [23].

Bidirectional MR analysis

We conducted bidirectional MR analysis to investigate the presence of reverse causality among associations between T2D-related traits and outcomes of interest. This was performed by switching the exposure and outcomes in opposite directions.

MR power analysis

Power calculations were conducted at https://sb452.shinyapps.io/power/ [79]. The proportion of variance in the exposure explained by the genetic variants (R^2) were required for MR power analysis, with 0.048 (FPG), 0.017 (2h-PG), and 0.024 (HbA1c) used, respectively. We assumed odds ratios (ORs) of 1.1 and 1.2 for binary outcomes and changes in outcomes in standard deviation (SD) units per SD change in exposure (0.1 and 0.2) for continuous outcomes. Statistical power evaluations at the conservative significance level [0.007 (Bonferroni correction with 7 tests)] are plotted in Figure 2.2.

Table 2.2. Recommended MR methods by assumption of IVs

No weak IVs (F>10)	NOME	No Heterogeneity	Recommended Methods
Satisfied	Satisfied	Satisfied	IVW
Satisfied	Satisfied	At least one violated	→Table 2.3
Satisfied	Violated	Satisfied	IVW
Satisfied	Violated	At least one violated	MR-Egger (SIMEX)
Violated	Satisfied	Satisfied	Weighted median
Violated	Satisfied	At least one violated	→Table 2.3
Violated	Violated	Satisfied	MR-Egger (SIMEX)
Violated	Violated	At least one violated	MR-Egger (SIMEX)

IV, instrument variable; NOME, NO Measurement Error; IVW, inverse−variance−weighted; SIMEX, simulation extrapolation;

Table 2.3. Recommended MR methods by heterogeneity test

Q test†	Q' test†	MR-PRESSO global test‡	Recommended Methods
			IVW
✓	✓		MR-Egger
	✓		IVW
✓			MR-Egger
		✓	IVW, if there is no weak IV MR-PRESSO, otherwise
✓	✓	✓	MR-PRESSO
	✓	✓	IVW, if there is no weak IV (F>10) MR-PRESSO, ow (F<10)
✓		✓	MR-Egger, MR-PRESSO

✓: significant (heterogeneity exists)

†Greco et al., 2015; Bowden et al., 2017

‡Verbanck et al., 2018

IV, instrument variable; IVW, inverse−variance−weighted; MR-PRESSO, MR-Plei otropy RESidual Sum and Outlier

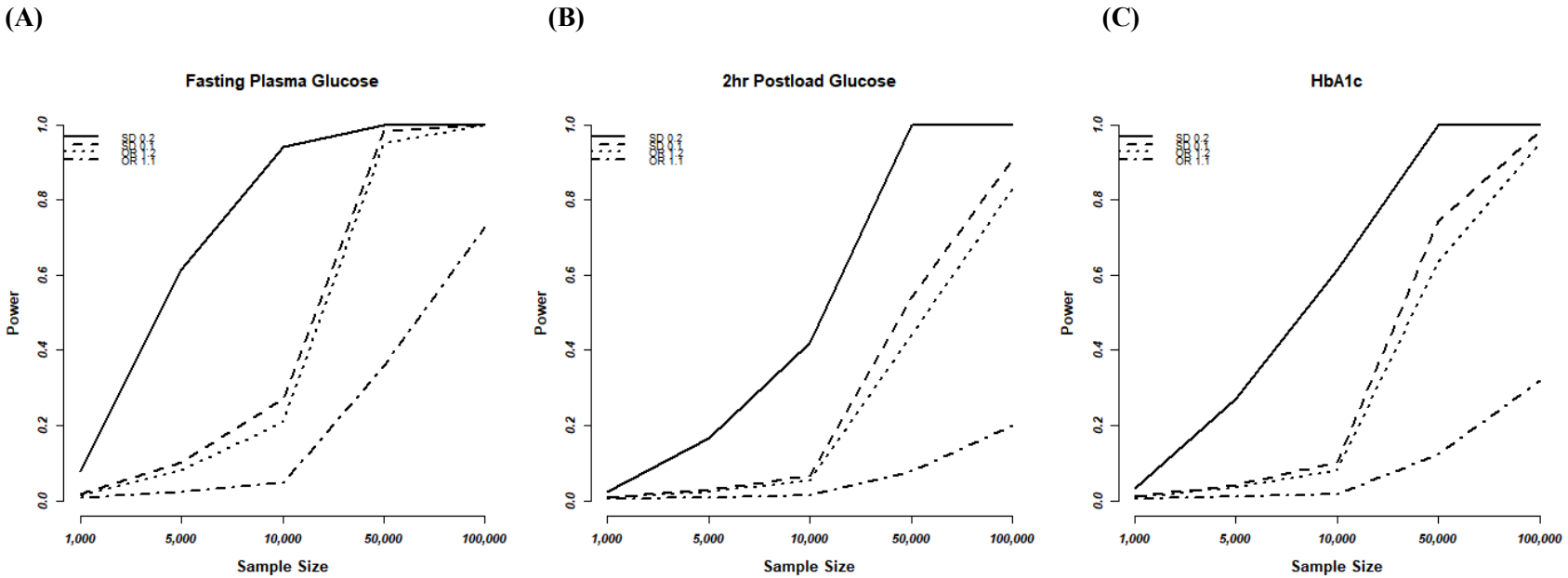


Figure 2.2. Statistical power evaluations of MR analyses based on the T2D-diagnosis criteria. (A) FPG, (B) 2h-PG, and (C) HbA1c. We used a conservative significance threshold of $P < 0.007$ with Bonferroni correction using 7 of testing.

2.3. Results

A total of 34, 7, and 11 genetic variants associated with FPG, 2h-PG, and HbA1c respectively, were available as potential instruments from studies included in MAGIC. Each IV set showed genome-wide significant ($P < 5 \times 10^{-8}$) associations with T2D-related traits and were not in LD or within 10,000 kb of an established signal. To investigate IV quality, we generated F-statistics, I^2 values, and *P*-values for Cochran's Q statistic from IVW, Rucker's Q' statistic from MR-Egger, and MR-PRESSO global test (Table 2.4). All instruments used for MR analyses had F-statistics >10, indicating no evidence of weak instrument bias. Rejection of the null hypothesis of the Cochran's Q statistic for heterogeneity suggested potential pleiotropy in the genetic variants and did not indicate that the InSIDE assumptions were invalid. When the pleiotropic effect was present, MR-Egger (with and without SIMEX), and MR-PRESSO) were performed rather than using the IVW method. The instruments corresponding to FPG satisfied the NOME assumption ($I^2 > 90$) but only partially satisfied this for HbA1c ($I^2 > 90$ in only some cases) and did not satisfy this in the case of 2h-PG ($I^2 < 90$). When the NOME assumption was violated, the results of MR-Egger (SIMEX) were generated. Using these IVs, we performed MR analyses for a total of 14 human health phenotypes, with all results (3 exposures × 13 phenotypes × 5 methods = 185 results) presented in Table 2.5. Application of Bonferroni correction to each disease category (0.05/6 = 0.008 for cancer; and 0.05/7 = 0.007 for vascular disease) revealed two significant phenotypes (CAD and LDL level) associated with T2D-related traits (Table 2.6). Additionally, we confirmed these relationships through bidirectional and replication analyses (Tables 2.6/ 2.7).

Table 2.4. Assumption check for instrumental variables.

	FPG (R^2 = 0.048)				2h-PG (R^2 = 0.017)				HbA1c (R^2 = 0.024)			
	N	**F**	I^2 (%)	Q-*P**	**N**	**F**	I^2 (%)	Q-*P*	**N**	**F**	I^2 (%)	Q-*P*
1. Cancer												
Breast cancer	34	130.5	96.8	<0.001	7	43.5	29.8	0.222	11	77.6	92.2	0.110
Lung cancer	33	133.0	97.3	0.182	7	43.5	36.2	0.322	11	77.6	87.6	0.021
SC lung cancer	33	43.5	40.1	0.599	7	43.5	40.1	0.875	11	77.6	87.9	0.198
Ovarian cancer	33	133.5	97.4	0.530	7	43.5	28.1	0.323	11	77.6	87.8	0.243
Pancreatic cancer	25	111.7	96.4	0.138	6	45.0	31.8	0.710	8	82.1	90.9	0.239
Thyroid cancer	27	144.9	97.9	0.183	5	47.4	21.9	0.434	9	81.7	91.2	0.882
2. Cardiovascular disease												
CAD	34	130.5	97.3	<0.001	7	43.5	23.0	<0.001	11	77.6	88.0	0.002
CKD	32	135.8	97.4	0.106	7	43.5	29.1	0.482	10	82.1	86.5	0.632
HDL cholesterol	34	130.5	97.3	<0.001	7	43.5	2.4	<0.001	11	77.6	90.9	0.545
LDL cholesterol	34	130.8	97.2	<0.001	7	43.5	5.7	<0.001	11	77.6	91.1	<0.001
Stroke	34	130.5	97.3	<0.001	7	43.5	39.9	0.069	11	77.6	86.0	0.075
Cardio-embolic	34	130.5	97.4	0.498	7	43.4	35.2	0.733	11	77.6	88.7	0.255
small-vessel	32	135.8	97.6	0.038	7	43.5	55.6	0.675	10	76.88	86.6	0.556

F, F-statistic; Q-P, P-value for the Q-statistic; SC, squamous cell; CKD, chronic kidney disease; CAD, coronary artery disease; LDL, low-density lipoprotein; HDL, high-density lipoprotein; FPG, fasting plasma glucose; 2h-PG, 2-h postload glucose; HbA1, hemoglobin A1c.

Table 2.5. MR results for T2D-related traits on cancers and vascular disease.

Trait and MR methods	Parameter	FPG N	FPG Estimate (95% CI)	FPG *P*	2h-PG N	2h-PG Estimate (95% CI)	2h-PG *P*	HbA1c N	HbA1c Estimate (95% CI)	HbA1c *P*
1. Cancer										
Breast cancer										
IVW	Estimate	34	−0.05 (−0.28, 0.18)	0.671	7	0.16 (−0.06, 0.37)	0.151	11	−0.01 (−0.28, 0.25)	0.922
MR-Egger	Intercept		0.00 (−0.01, 0.01)	0.537		−0.09 (−0.16, −0.01)	0.028		0.02 (0.00, 0.04)	0.067
	Slope		−0.18 (−0.65, 0.29)	0.456		1.08 (0.24, 1.92)	0.012		−0.46 (−0.98, 0.07)	0.092
MR-Egger (SIMEX)	Intercept		0.00 (−0.01, 0.01)	0.996		0.00 (−0.02, 0.03)	0.941		0.01 (−0.01, 0.02)	0.301
	Slope		−0.05 (−0.29, 0.19)	0.679		0.15 (−0.11, 0.42)	0.303		−0.04 (−0.30, 0.23)	0.800
MR-PRESSO (O-C)	Estimate	33	−0.10 (−0.32, 0.36)	0.364	5	0.16 (−0.01, 0.34)	0.068		No outlier	—
Weighted median	Estimate		−0.20 (−0.44, 0.04)	0.094		0.21 (0.06, 0.36)	0.008		−0.03 (−0.32, 0.29)	0.842
Lung cancer										
IVW	Estimate	33	0.05 (−0.22, 0.32)	0.721	7	−0.13 (−0.32, 0.06)	0.188	11	0.36 (−0.16, 0.88)	0.173
MR-Egger	Intercept		0.01 (−0.01, 0.02)	0.330		−0.06 (−0.15, 0.03)	0.182		0.04 (−0.01, 0.08)	0.102
	Slope		−0.17 (−0.68, 0.35)	0.523		0.48 (−0.43, 1.39)	0.301		−0.50 (−1.65, 0.64)	0.389
MR-Egger (SIMEX)	Intercept		−0.01 (−0.02, 0.01)	0.148		0.00 (−0.02, 0.03)	0.795		0.00 (−0.02, 0.02)	0.898
	Slope		0.14 (−0.19, 0.35)	0.351		−0.15 (−0.39, 0.09)	0.286		0.37 (−0.19, 0.94)	0.233
MR-PRESSO (O-C)	Estimate		No outlier	—		No outlier	—		No outlier	—
Weighted median	Estimate		−0.03 (−0.37, 0.32)	0.880		−0.08 (−0.32, 0.16)	0.494		0.04 (−0.47, 0.54)	0.886
SC lung cancer										
IVW	Estimate	33	0.39 (0.02, 0.76)	0.037	7	−0.19 (−0.46, 0.08)	0.161	11	0.51 (−0.12, 1.15)	0.115
MR-Egger	Intercept		0.00 (−0.02, 0.02)	0.792		0.00 (−0.13, 0.13)	0.968		0.01 (−0.05, 0.07)	0.651
	Slope		0.31 (−0.38, 1.00)	0.376		−0.17 (−1.52, 1.19)	0.811		0.18 (−1.38, 1.75)	0.818
MR-Egger (SIMEX)	Intercept		0.00 (−0.01, 0.01)	0.480		0.01 (−0.01, 0.03)	0.307		−0.01 (−0.04, 0.02)	0.453
	Slope		0.41 (0.05, 0.77)	0.032		−0.26 (−0.46, −0.06)	0.052		0.58 (−0.09, 1.26)	0.127
MR-PRESSO (O-C)	Estimate		No outlier	—		No outlier	—		No outlier	—
Weighted median	Estimate		0.36 (−0.17, 0.88)	0.180		−0.16 (−0.50, 0.17)	0.341		0.27 (−0.49, 1.03)	0.493
Ovarian cancer										
IVW	Estimate	33	−0.14 (−0.68, 0.42)	0.632	7	0.14 (−0.29, 0.58)	0.526	11	0.16 (−0.73, 1.06)	0.719
MR-Egger	Intercept		0.08 (−0.96, 1.13)	0.882		−0.01 (−0.24, 0.21)	0.905		0.06 (−0.02, 0.13)	0.142
	Slope		−0.01 (−0.04, 0.02)	0.637		0.28 (−2.12, 2.69)	0.817		−1.19 (−3.18, 0.80)	0.242
MR-Egger (SIMEX)	Intercept		−0.01 (−0.02, 0.01)	0.426		−0.04 (−0.07, −0.01)	0.044		0.03 (0.00, 0.06)	0.089
	Slope		−0.11 (−0.66, 0.44)	0.705		0.39 (0.03, 0.75)	0.087		−0.03 (−0.85, 0.79)	0.948
MR-PRESSO (O-C)	Estimate		No outlier	—		No outlier	—		No outlier	—
Weighted median	Estimate		−0.46 (−1.33, 0.40)	0.293		−0.01 (−0.54, 0.53)	0.980		0.08 (−1.05, 1.21)	0.886
Pancreatic cancer										
IVW	Estimate	25	−0.14 (−1.07, 0.79)	0.768	6	−0.01 (−0.50, 0.49)	0.973	8	0.53 (−0.64, 1.71)	0.374
MR-Egger	Intercept		0.02 (−0.03, 0.06)	0.537		−0.04 (−0.27, 0.18)	0.702		0.03 (−0.08, 0.14)	0.592
	Slope		−0.64 (−2.49, 1.21)	0.497		0.45 (−1.94, 2.84)	0.713		−0.11 (−2.78, 2.56)	0.934
MR-Egger (SIMEX)	Intercept		−0.01 (−0.04, 0.02)	0.455		0.00 (−0.01, 0.05)	0.874		0.01 (−0.04, 0.06)	0.792

	Slope		−0.07 (−1.03, 0.89)	0.883		0.02 (−0.53, 0.57)	0.942		0.51 (−0.77, 1.79)	0.464
MR-PRESSO (O-C)	Estimate		No outlier	—		No outlier	—		No outlier	—
Weighted median	Estimate		−0.81 (−2.01, 0.38)	0.184		−0.05 (−0.67, 0.57)	0.883		0.46 (−0.87, 1.78)	0.500
Thyroid cancer										
IVW	Estimate	27	−0.34 (−1.67, 0.98)	0.612	5	−0.53 (−1.43, 0.38)	0.257	9	0.68 (−0.97, 2.33)	0.417
MR-Egger	Intercept		0.06 (−0.01, 0.13)	0.116		0.32 (−0.08, 0.73)	0.122		−0.07 (−0.21, 0.07)	0.349
	Slope		−1.89 (−4.22, 0.43)	0.110		−3.97 (−8.43, 0.49)	0.081		2.30 (−1.47, 6.07)	0.231
MR-Egger (SIMEX)	Intercept		0.00 (−0.05, 0.04)	0.918		0.06 (−0.04, 0.17)	0.333		−0.02 (−0.07, 0.02)	0.344
	Slope		−0.34 (−1.73, 1.05)	0.641		−0.98 (−2.12, 0.16)	0.192		0.89 (−0.30, 2.09)	0.186
MR-PRESSO (O-C)	Estimate		No outlier	—		No outlier	—		No outlier	—
Weighted median	Estimate		−1.14 (−2.73, 0.44)	0.157		−0.80 (−1.98, 0.38)	0.184		1.19 (−0.89, 3.28)	0.262
2. Cardiovascular disease										
CAD										
IVW	Estimate	34	0.21 (0.05, 0.37)	0.012	7	0.12 (−0.06, 0.31)	0.183	11	0.24 (0.02, 0.46)	0.031
MR-Egger	Intercept		0.00 (−0.01, 0.01)	0.530		0.05 (−0.03, 0.14)	0.231		0.02 (−0.01, 0.03)	0.078
	Slope		0.13 (−0.18, 0.43)	0.426		−0.42 (−1.32, 0.48)	0.365		−0.14 (−0.60, 0.33)	0.569
MR-Egger (SIMEX)	Intercept		0.00 (−0.01, 0.01)	0.443		0.00 (−0.02, 0.02)	0.833		0.00 (−0.01, 0.01)	0.762
	Slope		0.22 (0.06, 0.39)	0.014		0.14 (−0.09, 0.37)	0.301		0.23 (−0.01, 0.47)	0.085
MR-PRESSO (O-C)	Estimate	32	0.18 (0.01, 0.35)	0.045	5	0.21 (0.13, 0.29)	<0.001	10	0.19 (−0.01, 0.39)	0.069
Weighted median	Estimate		0.29 (0.14, 0.45)	<0.001		0.21 (0.10, 0.31)	<0.001		0.09 (−0.11, 0.30)	0.369
CKD										
IVW	Estimate	32	0.12(−0.13, 0.36)	0.351	7	0.08 (−0.07, 0.23)	0.301	10	0.16 (−0.16, 0.47)	0.337
MR-Egger	Intercept		−0.01(−0.02, 0.01)	0.281		0.01 (−0.06, 0.09)	0.760		−0.01 (−0.03, 0.02)	0.697
	Slope		0.33(−0.13, 0.78)	0.159		−0.04 (−0.83, 0.75)	0.916		0.29 (−0.48, 1.07)	0.455
MR-Egger (SIMEX)	Intercept		0.01 (−0.01, 0.01)	0.109		0.01 (−0.01, 0.02)	0.555		0.00 (−0.01, 0.01)	0.785
	Slope		0.08 (−0.16, 0.32)	0.496		0.05 (−0.13, 0.23)	0.601		0.17 (−0.14, 0.49)	0.315
MR-PRESSO (O-C)	Estimate		No outlier	—		No outlier	—		No outlier	—
Weighted median	Estimate		0.17(−0.12, 0.46)	0.252		0.09 (−0.10, 0.28)	0.359		0.24 (−0.17, 0.65)	0.243
HDL cholesterol										
IVW	Estimate	34	−0.08 (−0.08, 0.25)	0.320	7	0.07 (−0.08, 0.23)	0.342	11	0.19 (−0.11, 0.51)	0.206
MR-Egger	Intercept		0.01 (0.00, 0.02)	0.219		0.02 (−0.06, 0.09)	0.680		0.03 (0.00, 0.06)	0.049
	Slope		−0.08 (−0.39, 0.23)	0.606		−0.10 (−0.94, 0.74)	0.817		−0.37 (−1.01, 0.27)	0.252
MR-Egger (SIMEX)	Intercept		0.00 (−0.01, 0.01)	0.728		−0.01 (−0.03, 0.00)	0.064		0.01 (−0.01, 0.02)	0.227
	Slope		0.09 (−0.08, 0.26)	0.259		0.15 (0.02, 0.28)	0.074		0.17 (−0.12, 0.47)	0.282
MR-PRESSO (O-C)	Estimate	28	0.04 (−0.03, 0.09)	0.265	5	0.04 (−0.02, 0.11)	0.206		No outlier	—
Weighted median	Estimate		0.03 (−0.05, 0.10)	0.514		0.01 (−0.05, 0.06)	0.833		0.07 (−0.32, 0.46)	0.729
LDL cholesterol										
IVW	Estimate	34	0.02 (−0.16, 0.20)	0.807	7	0.04 (−0.11, 0.19)	0.626	11	0.23 (0.05, 0.41)	0.015
MR-Egger	Intercept		0.01 (−0.01, 0.01)	0.394		0.02 (−0.05, 0.09)	0.566		−0.01 (−0.03, 0.01)	0.281
	Slope		−0.10 (−0.44, 0.24)	0.551		−0.19 (−1.00, 0.61)	0.636		0.45 (0.01, 0.88)	0.046

MR-Egger (SIMEX)	Intercept		0.00 (−0.01, 0.01)	0.341		−0.01 (−0.03, 0.01)	0.143		−0.01 (−0.02, 0.00)	0.053
	Slope		0.04 (−0.14, 0.22)	0.695		0.10 (−0.05, 0.25)	0.241		0.27 (0.11, 0.44)	0.007
MR-PRESSO (O-C)	Estimate	27	0.04 (−0.03, 0.11)	0.225	5	0.06 (0.01, 0.11)	0.033	10	0.14 (0.03, 0.25)	0.010
Weighted median	Estimate		−0.01 (−0.08, 0.07)	0.890		0.04 (−0.02, 0.10)	0.214		0.15 (0.03, 0.26)	0.012
Stroke										
IVW	Estimate	34	0.15 (−0.03, 0.33)	0.104	7	0.06 (−0.06, 0.17)	0.338	11	−0.06 (−0.27, 0.15)	0.567
MR-Egger	Intercept		0.01 (0.00, 0.02)	0.005		−0.02 (−0.07, 0.04)	0.579		0.01 (−0.01, 0.03)	0.308
	Slope		−0.24 (−0.56, 0.08)	0.137		0.22 (−0.37, 0.81)	0.467		−0.30 (−0.81, 0.21)	0.244
MR-Egger (SIMEX)	Intercept		0.00 (−0.01, 0.01)	0.174		0.00 (−0.01, 0.01)	0.930		0.00 (−0.01, 0.01)	0.396
	Slope		0.17 (−0.01, 0.35)	0.078		0.06 (−0.09, 0.21)	0.469		−0.09 (−0.31, 0.13)	0.451
MR-PRESSO (O-C)	Estimate	33	0.13 (−0.04, 0.31)	0.135		No outlier	—		No outlier	—
Weighted median	Estimate		0.02 (−0.16, 0.19)	0.835		0.04 (−0.08, 0.16)	0.506		−0.15 (−0.38, 0.08)	0.205
Stroke (cardio-embolic)										
IVW	Estimate	34	0.22 (−0.05, 0.48)	0.118	7	0.06 (−0.13, 0.26)	0.530	11	−0.51 (−0.94, −0.04)	0.023
MR-Egger	Intercept		0.01 (0.00, 0.03)	0.047		0.00 (−0.09, 0.09)	0.988		0.02 (−0.02, 0.06)	0.369
	Slope		−0.23 (−0.74, 0.29)	0.387		0.07 (−0.91, 1.05)	0.889		−0.93 (−1.96, 0.09)	0.076
MR-Egger (SIMEX)	Intercept		0.00 (−0.01, 0.01)	0.266		−0.01 (−0.02, 0.01)	0.383		0.01 (−0.01, 0.02)	0.403
	Slope		−0.03 (−0.03, 0.51)	0.094		0.11 (−0.07, 0.29)	0.294		−0.56 (−1.01, −0.10)	0.039
MR-PRESSO (O-C)	Estimate		No outlier	—		No outlier	—		No outlier	—
Weighted median	Estimate		0.03 (−0.36, 0.41)	0.889		0.01 (−0.24, 0.25)	0.937		−0.55 (−1.09, −0.02)	0.045
Stroke (small-vessel)										
IVW	Estimate	32	0.34 (0.04, 0.64)	0.025	7	0.16 (−0.02, 0.33)	0.084	10	0.37 (0.01, 0.73)	0.046
MR-Egger	Intercept		0.02 (0.01, 0.04)	0.016		0.03 (−0.05, 0.11)	0.443		−0.01 (−0.05, 0.02)	0.422
	Slope		−0.22 (−0.75, 0.32)	0.427		−0.16 (−1.00, 0.67)	0.700		0.71 (−0.19, 1.61)	0.124
MR-Egger (SIMEX)	Intercept		−0.01 (−0.01, 0.01)	0.209		0.00 (−0.02, 0.02)	0.744		0.00 (−0.02, 0.01)	0.551
	Slope		0.37 (0.08, 0.68)	0.020		0.11 (−0.07, 0.34)	0.255		0.42 (0.04, 0.79)	0.063
MR-PRESSO (O-C)	Estimate		No outlier	—		No outlier	—		No outlier	—
Weighted median	Estimate		0.16 (−0.24, 0.55)	0.435		0.12 (−0.10, 0.35)	0.294		0.46 (−0.03, 0.94)	0.065

MR, Mendalian randomization; T2D, type 2 diabetes; IVW, inverse−variance−weighted; SIMEX, simulation extrapolation; PRESSO (O-C), Pleiotropy RESidual Sum and Outlier (outlier-correction); SC, squamous cell; LDL, low-density lipoprotein; HDL, high-density lipoprotein; CKD, chronic kidney disease; CAD, coronary artery disease; CI, confidence interval; FPG, fasting plasma glucose; 2h-PG, 2-h postload glucose; Hb1Ac, hemoglobin A1c.

Table 2.6. Significant results from MR and replication analyses

No.	Exposure	Outcome	Original study					Replication study		
			MR method	Parameter	N	Estimate (95% CI)	*P*	N	Estimate (95% CI)	*P*
1	FPG	CAD	IVW	Estimate	34	0.21 (0.05, 0.37)	0.012	34	0.14 (−0.02, 0.29)	0.078
			MR Egger	Intercept		0.00 (−0.01, 0.01)	0.530		0.01 (0.00, 0.02)	0.090
				Slope		0.13 (−0.18, 0.43)	0.426		−0.07 (−0.36, 0.22)	0.626
			MR Egger (SIMEX)	Intercept		0.00 (−0.01, 0.01)	0.443		0.00 (−0.01, 0.01)	0.769
				Slope		0.22 (0.06, 0.39)	0.014		0.144 (−0.02, 0.30)	0.087
			MR-PRESSO (O-C)	Estimate	32	0.18 (0.01, 0.35)	0.045	28	0.19 (0.07, 0.32)	0.002
			Weighted median	Estimate		0.29 (0.14, 0.45)	<0.001		0.20 (0.07, 0.34)	0.003
2	HbA1c	LDL cholesterol	IVW	Estimate	11	0.23 (0.05, 0.41)	0.015	10	0.15 (−0.31, 0.61)	0.521
			MR Egger	Intercept		−0.01 (−0.03, 0.01)	0.281		−0.05(−0.08, −0.01)	0.004
				Slope		0.45 (0.01, 0.88)	0.046		0.14 (0.39, 1.89)	0.003
			MR Egger (SIMEX)	Intercept		−0.01 (−0.02, 0.00)	0.053		0.00 (−0.02, 0.02)	0.780
				Slope		0.27 (0.11, 0.44)	0.007		0.16 (−0.33, 0.65)	0.539
			MR-PRESSO (O-C)	Estimate	10	0.14 (0.03, 0.25)	0.010	9	0.38 (0.03, 0.72)	0.032
			Weighted median	Estimate		0.15 (0.03, 0.26)	0.012		0.44 (0.03, 0.84)	0.036

MR, Mendalian randomization; IVW, inverse−variance−weighted; SIMEX, simulation extrapolation; PRESSO (O-C), Pleiotropy RESidual Sum and Outlier (outlier-correction); LDL, low-density lipoprotein; CAD, coronary artery disease; CI, confidence interval; FPG, fasting plasma glucose; Hb1Ac, hemoglobin A1c.

Table 2.7. Significant results from bidirectional MR analysis

No.	Exposure	Outcome			Original study			Replication study	
			MR methods	parameter	N	Estimate (95% CI)	*P*	N	Estimate (95% CI)	*P*
1	CAD	FPG	IVW	Estimate	30	0.00 (−0.02, 0.02)	0.873	83	0.00 (−0.01, 0.01)	0.834
			MR Egger	Intercept		0.00 (−0.01, 0.01)	0.352		0.00 (−0.01, 0.01)	0.813
				Slope		−0.02 (−0.06, 0.02)	0.437		0.00 (−0.02, 0.03)	0.906
			MR Egger (SIMEX)	Intercept		0.00 (−0.01, 0.01)	0.435		0.00 (−0.01, 0.01)	0.907
				Slope		0.01 (−0.01, 0.02)	0.781		0.00 (−0.01, 0.01)	0.844
			MR-PRESSO (O-C)	Estimate	29	0.00 (−0.02, 0.02)	0.877		No outlier	—
			Weighted median	Estimate		0.01 (−0.01, 0.03)	0.186		0.00 (−0.01, 0.01)	0.834
2	LDL cholesterol	HbA1c	IVW	Estimate	74	0.02 (−0.01, 0.05)	0.202	4	−0.01 (−0.04, 0.03)	0.681
			MR Egger	Intercept		0.00 (−0.01, 0.01)	0.263		0.00 (−0.02, 0.02)	0.796
				Slope		−0.01 (−0.06, 0.05)	0.859		−0.02 (−0.13, 0.09)	0.719
			MR Egger (SIMEX)	Intercept		0.02 (−0.01, 0.00)	0.056		0.00 (−0.01, 0.01)	0.513
				Slope		0.02 (−0.01, 0.05)	0.158		−0.02 (−0.03, 0.01)	0.548
			MR-PRESSO (O-C)	Estimate	71	0.02 (−0.01, 0.04)	0.234		Not enough IVs	—
			Weighted median	Estimate		−0.01 (−0.03, 0.03)	0.911		−0.02 (−0.04, 0.02)	0.376

MR, Mendalian randomization; IVW, inverse−variance−weighted; SIMEX, simulation extrapolation; PRESSO (O-C), Pleiotropy RESidual Sum and Outlier (outlier-correction); LDL, low-density lipoprotein; CAD, coronary artery disease; CI, confidence interval; FPG, fasting plasma glucose; Hb1Ac, hemoglobin A1c.

T2D-related traits and cancers

We considered FPG, 2h-PG, and HbA1c as exposure traits. For FPG, IVs for lung, ovarian, pancreatic, and thyroid cancer satisfied the IV assumptions (F statistics >10, $I^2 > 90$, Q-*P*>0.05), and IVW was selected for MR analyses (Table 2.4). No significant causal association was observed between FPG and lung (*P*=0.721), ovarian (*P*=0.632), pancreatic (*P*=0.768), and thyroid (*P*=0.612) cancer. A pleiotropic effect was observed in breast cancer through Q (*P*<0.001), Q' (*P*<0.001) statistics, and the MR-PRESSO global test (*P*<0.001), and MR-PRESSO did not yield significant outcomes (*P*=0.364). The NOME assumption was violated in SC lung cancer (I^2 <90), and the MR-Egger (SIMEX) method was used. The MR-Egger (SIMEX) method yielded nominally significant (*P*<0.05) causal effects (*P*=0.032). Furthermore, when 2h-PG was considered an exposure trait, IVs for all cancers, except for breast cancer, we found no weak instrument bias (F>10) and no heterogeneity (Q-*P*>0.05, Q'-*P*>0.05, MR-PRESSO global test-*P*>0.05), and the IVW method was used. However, IVs for breast cancer have a measurement error (I^2 <90), and the MR-Egger (SIMEX) method was used. None of the IVs were significant for breast (*P*=0.303), lung (*P*=0.721), SC lung (*P*=0.037), ovarian (*P*=0.632), pancreatic (*P*=0.768), and thyroid (*P*=0.612) cancer. Moreover, regarding HbA1c, evidence of violations of IV assumptions for all cancers was obtained (F statistics >10, Q-*P*>0.05, Q'-*P*>0.05, MR-PRESSO global test-*P*>0.05), and IVW was applied. No significant association was observed between HbA1c and breast (P=0.922), lung (*P*=0.173), SC lung (*P*=0.115), ovarian (*P*=0.719), pancreatic (*P*=0.374), and thyroid (*P*=0.417) cancer.

For lung, breast, and ovarian cancer, we assumed an OR of 1.2 and we determined the statistical power at between 40% and 70%. The highest power was observed for FPG with the highest R^2, followed by HbA1c and 2h-PG. The estimated statistical power was the highest (>80%) for SC lung cancer for all T2D-related traits owing to a sample size of >100,000

individuals if the standardized effect size is assumed to be same. However, for pancreatic and thyroid cancers, the sample size was small (3,835 and 1,080, respectively), thus decreasing the statistical power, indicating the possibility of false-negative results. The overall estimated power (Figure 2.2) revealed no causal effect of FPG, 2h-PG, and HbA1c on breast, lung, SC lung, ovarian, pancreatic, and thyroid cancers ($P < 0.008$ after Bonferroni correction) (Table 2.5).

T2D-related traits and vascular diseases

All data for vascular diseases were from a sample size of >100,000 patients, giving them a power of ≥80%, except for detecting an OR of 1.1. We found no causal effect of FPG, 2h-PG, or HbA1c on CKD, HDL level, stroke, or stroke subtype, but two significant causal relationships were observed for FPG with CAD and HbA1c with HDL level. Interestingly, three T2D-related traits used as criteria for diagnosing T2D showed different results for the same phenotype. First, on using FPG as an exposure trait, IVs for CKD and cardio-embolic stroke strongly satisfied the IV assumptions (F statistics >10, $I^2 > 90$, F statistics >10, Q-*P*>0.05, Q'-*P*>0.05, MR-PRESSO global test-*P*>0.05), and the IVW approach was selected (Table 2.2). However, FPG had no causal effects on CKD (*P*=0.351) and cardio-embolic stroke (*P*=0.118) were observed, but was nominally significant on small-vessel stroke (*P*=0.025). In the case of CAD, HDL/LDL cholesterol, and stroke, we found heterogeneity (Q-*P*<0.05, Q'-*P*<0.05, MR-PRESSO global test-*P*<0.05), and the MR-PRESSO method was applied. Nominally significant results were observed for CAD (MR-PRESSO *P*=0.045) and non-significant results were observed for HDL cholesterol (*P*=0.265), LDL cholesterol (*P*=0.225), and stroke (*P*=0.135). Second, when 2h-PG was used as an exposure trait, IVs for CKD, stroke, cardio-embolic stroke, and small-vessel stroke strongly satisfied the IV assumptions (F statistics >10, F statistics >10,

Q-*P*>0.05, Q'-*P*>0.05, MR-PRESSO global test-*P*>0.05), and the IVW method used. Non-significant causal effects were observed for 2h-PG on CKD (*P*=0.183), stroke (*P*=0.338), cardio-embolic stroke (*P*=0.530), and small-vessel stroke (*P*=0.084). In the case of CAD, HDL/LDL cholesterol, all have measurement error (I^2 <90) with heterogeneity (Q-*P*<0.05, Q'-*P*<0.05, MR-PRESSO global test-*P*<0.05), and the MR-Egger (SIMEX) method was used. Non-significant causal effects were observed for 2h-PG on CAD (*P*=0.301), HDL cholesterol (*P*=0.074), and LDL cholesterol (*P*=0.241). Third, when HbA1c was considered an exposure trait, IVs for CKD, HDL cholesterol, stroke, cardio-embolic stroke and small-vessel stroke strongly satisfied the IV assumptions (F statistics >10, Q-*P*>0.05, Q'-*P*>0.05, MR-PRESSO global test-*P*>0.05), and the IVW method was selected. However, no causal effects of HbA1c were observed on CKD (*P*=0.337), HDL cholesterol (*P*=0.206), and stroke (*P*=0.567), but there were nominally significant implications for cardio-embolic stroke (*P*=0.023) and small-vessel stroke (*P*=0.046). Owing to the heterogeneity in CAD and LDL cholesterol (Q-*P*<0.05, Q'-*P*<0.05, MR-PRESSO global test-*P*<0.05), the MR-PRESSO method was considered, and nominally significant results were obtained for LDL cholesterol (*P*=0.010), but non-significant for CAD (*P*=0.069).

Significant effects were found for FPG-CAD and HbA1c-LDL cholesterol. Regarding FPG-CAD, all SNP-exposure and SNP-outcome effects are presented in Table 3.8. We found two SNPs significantly correlated with CAD (rs1260326: $P = 2.40 \times 10^{-5}$; and rs7651090: $P = 1.20 \times 10^{-5}$); however, given that they exhibited balanced pleiotropy, they were not excluded from the analysis (but were excluded from MR-PRESSO tests). A generated funnel plot showed symmetry, indicating heterogeneity due to horizontal pleiotropy (Figure 2.3A). The associations of the variants with FPG and CAD are shown in a scatter plot with five MR-fitted lines (Figure 2.3B). Thirty-four SNPs were considered instruments, and no weak instrument bias was noted

with no violation of NOME assumption, albeit with heterogeneity. Therefore, we assessed the other several sensitivity methods, and observed causal effects of CAD on FPG from the weighted median ($P < 0.001$). Moreover, we verified that reverse causality did not exist (Table 2.7). In the replication study using the same IVs and different GWAS data for outcome (PmID = 29212778, N = 296,525, P = European, and unit = logOR), there was no weak instrument bias of IVs (N=34, F statistics 43.5) but the heterogeneity assumption was violated (Q-P<0.05, Q'-P<0.05, MR-PRESSO global test-P<0.05). Therefore, MR-PRESSO was selected, and we found that FPG has a positively causal effect on CAD in accordance with the MR-PRESSO method ($P = 0.002$) (Table 2.6). On bidirectional MR analysis in the replication study, 83 SNPs were considered instrument variables. Weak instrument bias (F-statistics 77.2) and the NOME assumption (I^2 =92.4) were preserved; however, heterogeneity was observed Q-P<0.05, Q'-P < 0.05, MR-PRESSO global test-P<0.05). MR-PRESSO revealed no causal effect of CAD on FPG (Table 2.7).

Regarding HbA1c and LDL cholesterol, SNP-exposure and SNP-outcome effects (Table 2.9) indicated that one SNP was significantly correlated with LDL level (rs1800562; P=4.42 × 10^{-4}), with this SNP excluded from MR-PRESSO analysis. Figure 2.4A shows a funnel plot indicating slight non-symmetry, suggesting the presence of heterogeneity due to horizontal pleiotropy. The scatter plot in Figure 2.4B shows the associations of the variants with HbA1c and LDL level. Eleven SNPs were considered instruments, and no weak instrument bias was noted with no violation of NOME assumption, albeit with heterogeneity. Therefore, we assessed several other sensitivity methods, and observed causal effects of HbA1c on LDL cholesterol from MR-Egger (SIMEX) (P=0.007). In addition, reverse causality was not identified (Table 2.7). Seventy-four SNPs were considered instruments, and no weak instrument bias was noted (F-statistics 153.9), with no violation of the NOME assumption (I^2 =97.7).

However, heterogeneity was observed (Q-*P*<0.05, Q'-*P*<0.05, MR-PRESSO global test-*P* < 0.05), and MR-PRESSO revealed no causal effect of LDL cholesterol on HbA1c (*P*=0.234). Replication analysis using the same IVs and different GWAS data for the outcome-SNP effect (pmID = 28887542, N = 9,961, P = European, unit = mg/dL) revealed no evidence of a weak instrument bias (N=11, F-statistics 77.6) and no heterogeneity (Q-*P*>0.05, Q'-*P*>0.05, MR-PRESSO global test>0.05), but the NOME assumption (I^2 =87.9) was violated. Therefore, MR-PRESSO revealed significant results for the causal effect of HbA1c on LDL cholesterol (*P*=0.032) (Table 2.6). On bidirectional MR analysis for the replication study, 4 SNPs were considered instrument variables. No weak instrument bias (F-statistics 42.9) and no heterogeneity (Q-*P*>0.05, Q'-*P*>0.05, MR-PRESSO global test>0.05) were observed; however, a violation of the NOME assumption (I^2 =4) was noted. Accordingly, IVW was considered, and no causal effect of LDL cholesterol on HbA1c was observed (*P*=0.681) (Table 2.7).

Table 2.8. Instrument variables for FPG and CAD.

Chr	SNP	EA	OA	SNP Exposure			SNP Outcome		
				Beta	SE	*P*	Beta	SE	*P*
1	rs17712208	A	T	0.051	0.007	3.22×10^{-12}	0.002	0.019	0.920
2	rs1260326	C	T	0.029	0.002	2.17×10^{-41}	−0.030	0.007	2.4×10^{-05}
2	rs479661	A	G	−0.019	0.003	8.56×10^{-12}	−0.014	0.009	0.150
2	rs560887	C	T	0.071	0.003	1.40×10^{-178}	0.023	0.008	0.002
3	rs11708067	G	A	−0.023	0.003	1.30×10^{-18}	−0.020	0.008	0.017
3	rs11715915	T	C	−0.012	0.002	4.90×10^{-08}	−0.021	0.008	0.005
3	rs1280	C	T	−0.026	0.003	8.56×10^{-18}	0.007	0.010	0.520
3	rs7651090	G	A	0.013	0.002	1.75×10^{-08}	0.033	0.007	1.2×10^{-05}
5	rs4869272	T	C	0.018	0.002	1.02×10^{-15}	0.003	0.007	0.660
6	rs9368222	A	C	0.014	0.002	1.00×10^{-09}	−0.005	0.008	0.560
7	rs17168486	T	C	0.031	0.003	3.17×10^{-28}	−0.006	0.009	0.500
7	rs2191349	T	G	0.029	0.002	1.28×10^{-42}	0.011	0.007	0.130
7	rs6943153	C	T	−0.015	0.002	1.63×10^{-12}	0.005	0.007	0.500
7	rs6975024	C	T	0.061	0.003	2.88×10^{-99}	0.027	0.009	0.003
7	rs882020	T	C	0.021	0.003	3.04×10^{-12}	0.015	0.010	0.150
8	rs11558471	G	A	−0.029	0.002	7.80×10^{-37}	−0.003	0.008	0.709
8	rs983309	G	T	−0.026	0.003	6.29×10^{-15}	0.019	0.011	0.072
9	rs10811661	C	T	−0.024	0.003	5.65×10^{-18}	−0.008	0.009	0.410
9	rs10814916	C	A	0.016	0.002	2.26×10^{-13}	−0.010	0.007	0.130
9	rs16913693	G	T	−0.043	0.007	3.51×10^{-11}	−0.004	0.021	0.830
9	rs3829109	A	G	−0.017	0.003	1.13×10^{-10}	−0.025	0.008	0.002
10	rs11195502	T	C	−0.032	0.004	1.97×10^{-18}	−0.023	0.012	0.048
10	rs7903146	T	C	0.022	0.002	2.71×10^{-20}	0.017	0.008	0.027
11	rs10830963	G	C	0.078	0.003	1.00×10^{-200}	0.007	0.008	0.370
11	rs11603334	A	G	−0.019	0.003	1.12×10^{-11}	0.008	0.010	0.390
11	rs11607883	A	G	−0.021	0.002	6.32×10^{-24}	0.000	0.007	0.960
11	rs174576	A	C	−0.020	0.002	1.18×10^{-18}	−0.017	0.007	0.019
11	rs749067	C	T	−0.017	0.002	6.12×10^{-15}	0.001	0.007	0.940
12	rs10747083	A	G	0.013	0.002	7.57×10^{-09}	0.007	0.008	0.320
13	rs11619319	G	A	0.020	0.002	1.33×10^{-15}	0.008	0.008	0.320
14	rs3783347	T	G	−0.017	0.003	1.32×10^{-10}	−0.020	0.008	0.020
15	rs4502156	C	T	−0.022	0.002	1.38×10^{-25}	0.001	0.007	0.890
20	rs6072275	A	G	0.016	0.003	1.66×10^{-08}	−0.016	0.010	0.110
20	rs6113722	A	G	−0.035	0.005	2.49×10^{-11}	−0.012	0.018	0.500

FPG, fasting plasma glucose; CAD, coronary artery disease; Chr, chromosome; EA, effect all ele; OA, other allele; SE, standard error; LDL, low−density lipoprotein; HbA1, hemoglob in A1c; SNP, single-nucleotide polymorphism.

Table 2.9. Instrument variables for HbA1c and LDL cholesterol.

Chr	SNP	EA	OA	SNP Exposure			SNP Outcome		
				Beta	SE	*P*	Beta	SE	*P*
1	rs2779116	T	C	0.024	0.004	2.75×10^{-09}	0.013	0.006	0.106
2	rs552976	G	A	0.029	0.003	8.16×10^{-18}	0.002	0.004	0.424
6	rs1800562	A	G	−0.064	0.007	2.59×10^{-20}	−0.062	0.008	4.42×10^{-04}
7	rs1799884	T	C	0.038	0.004	1.45×10^{-20}	−0.002	0.005	0.988
8	rs4737009	A	G	0.027	0.004	6.12×10^{-12}	0.003	0.004	0.574
8	rs6474359	C	T	−0.060	0.011	1.18×10^{-08}	−0.026	0.011	0.030
10	rs16926246	T	C	−0.089	0.006	3.11×10^{-54}	−0.018	0.007	0.001
11	rs1387153	T	C	0.026	0.004	3.96×10^{-11}	−0.003	0.004	0.490
13	rs7998202	G	A	0.031	0.005	5.24×10^{-09}	−0.005	0.008	0.804
17	rs1046896	T	C	0.035	0.003	1.58×10^{-26}	0.002	0.004	0.528
22	rs855791	G	A	−0.027	0.004	2.74×10^{-14}	−0.010	0.004	0.003

Chr, chromosome; EA, effect allele; OA, other allele; SE, standard error; LDL, low−density lipoprotein; HbA1c, hemoglobin A1c; SNP, single-nucleotide polymorphism.

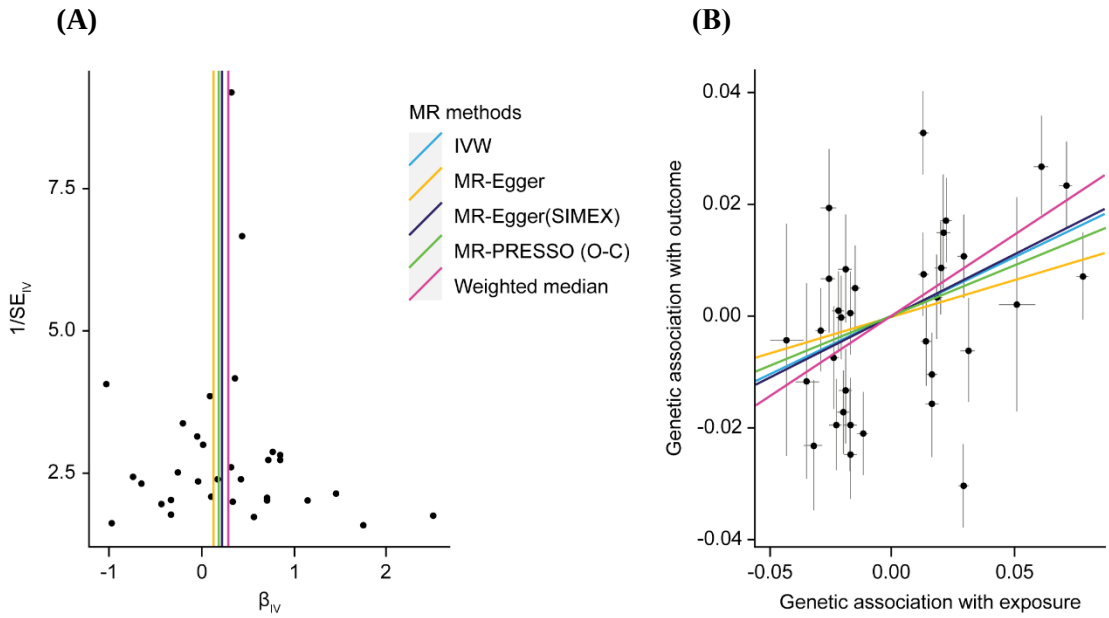


Figure 2.3. MR analysis of the effect of FPG on CAD. **(A)** Funnel plot displaying individual causal effect estimates for FPG on CAD. Dots representing the estimated causal effect for each IV. **(B)** The association between the effect size estimates on the FPG (X-axis) and CAD (Y-axis) for all SNPs that served as IVs.
FPG, fasting plasma glucose; CAD, coronary artery disease; IV, instrumental variable; SNP, single-nucleotide polymorphism; SIMEX, simulation extrapolation; PRESSO (O-C), Pleiotropy RESidual Sum and Outlier (outlier-correction).

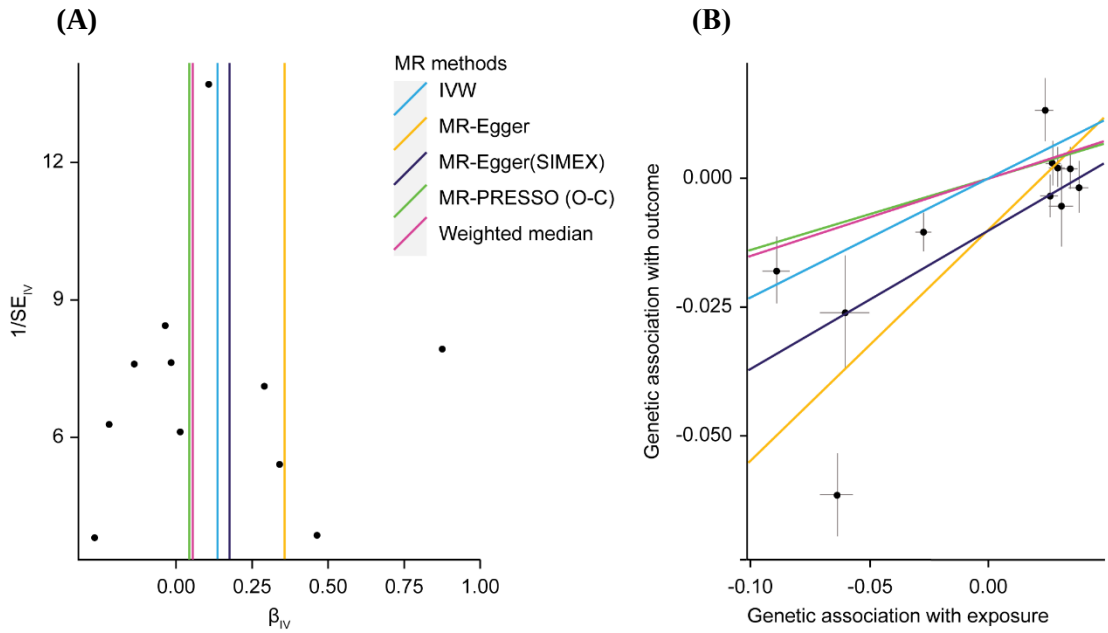


Figure 2.4. MR analysis of the effect of HbA1c on LDL levels. **(A)** Funnel plot displaying individual causal effect estimates for HbA1c on LDL levels. Dots represent the estimated causal effect for each IV. **(B)** The relationship between the effect size estimates on HbA1c (X-axis) and LDL level (Y-axis) for all SNPs that served as IVs.
MR, Mendalian randomization; HbA1c, hemoglobin A1c; LDL, low-density lipoprotein; IV, instrumental variable; SNP, single-nucleotide polymorphism; SIMEX, simulation extrapolation; PRESSO (O-C), Pleiotropy RESidual Sum and Outlier (outlier-correction).

2.4. Discussion

In this study, we performed MR analysis of the effect of T2D-related traits on 13 human health phenotypes using GWAS results and data from the MR-BASE registry. In particular, MR analysis was conducted according to three T2D-related criteria (FPG and 2h-PG from the OGTT and HbA1c). MR analyses reduce potential confounding effects and reverse causation, and our results are concurrent with those of previous epidemiological studies. Previous large meta-analyses or systematic reviews of epidemiological studies show that the association between T2D and cancer development is unclear [67]. Moreover, most epidemiological studies report limitations in findings of T2D-related association with cancers, because they were based on self-reported health assessments with high specificity (>90%) but low sensitivity (66%) as compared with medical records [83]. Recently, results of MR analysis indicated no strong evidence supporting a causal relationship between T2D and major solid tumors (stomach, colorectal, liver, pancreas, lung, breast, and prostate) [84]. Similarly, in the present study, analysis of European data from the MR-Base registry revealed no significant causal effect of T2D-related traits on breast, lung, SC lung, ovarian, pancreatic, and thyroid cancers. Although T2D and cancer share a number of risk factors, such as hyperglycemia, insulin resistance, and dyslipidemia, a relationship between the diseases has not been fully demonstrated [85]. Additionally, studies have reported correlations between hypoglycemic agents and cancer incidence, although these findings remain controversial [86, 87].

In T2D patients, the risk of death from cardiovascular disease increases along with elevated FPG and HbA1c levels, with HbA1c level correlated with microvascular and microvascular complications [83, 88, 89]. Therefore, hyperglycemia represents a strong independent factor for cardiovascular disease, with the risk increasing 2- to 3-fold in men and 3- to 4-fold women diagnosed with T2D relative to those without T2D [83, 88]. A longitudinal

study involving follow-up for 8 years of 2,363 non-diabetic adults between the ages of 50 and 75 years reported significant association between 2h-PG and HbA1c levels and an increased risk of death from cardiovascular disease [69]. Moreover, that study identified HbA1c level as not only predictive of improved better mortality from cardiovascular disease relative to FPG and 2h-PG [90] but also an independent risk factor for atherosclerosis and cardiovascular disease independent of T2D [91, 92]. In the present study, our findings indicated that vascular disease and LDL level were significantly linked with HbA1c level but not FPG or 2h-PG.

We found that different characteristics related to FPG, 2h-PG, and HbA1c differentially influenced IV characteristics. The 2h-PG results from an OGTT represent a standard test for T2D diagnosis. Although 2h-PG testing is more highly sensitive and specific than FPG testing, its low reproducibility is a disadvantage [93]. The low reproducibility is a consequence of changes in 2-h glucose concentrations for each measurement within a 48-h or 1-week time period in the same individual. On the other hand, FPG testing is simple and reproducible; however, the sensitivity for T2D diagnosis is poor, because it does not allow accurate identification of hyperglycemia after glucose load [94]. HbA1c reflects overall tissue protein glycation and can better reflect the overall biological effect of blood sugar as a 3-month average blood sugar estimate [95]; however, HbA1c measurements can be affected by hemoglobin disease, chronic renal failure, testing methods, and/or specific dosage [96]. Therefore, these findings suggest that the measurement error associated with SNP-exposure associations might be large when using any of these criteria. A previous study showed that calculation of the I^2 value confirmed the inadequacy of the NOME assumption due to measurement error related to 2h-PG testing [23]. Furthermore, reports indicated that the HbA1c level shows less variability in day-to-day within-person variance than FPG (<2% for HbA1c vs. 12–15% for FPG) [97], and the intra-individual coefficient of variation for FPG (6.4%) is less

than that for 2h-PG (16.7%) [98]. Therefore, MR analysis using 2h-PG as an exposure can be expected to increase the reliability of MR-Egger (SIMEX) findings relative to other methods. In the cases of FPG and HbA1c, IVW results and the sensitivity analysis methods should be examined more broadly.

We performed MR analysis using public data from previous large-scale GWAS studies. Producing in-house genetic data is expensive and requires substantial human resources, making it difficult for many individual researchers lacking access to appropriate datasets. A two-sample MR approach represents an effective method for discovering novel causal relationships through the use of available large-scale GWAS datasets. Additionally, MR analysis excludes confounding effects by using SNPs associated with exposure as genetic instruments, which also reduces the adverse effects of inaccurate data on hindering identification of relationships between exposure and outcome.

The present MR analysis has several limitations. First, some subjects may have overlapped between the two data sets with respect to the estimates of instrument-exposure and instrument-outcome, which could lead to inflated type 1 error rates and false-positive findings [43]. Furthermore, MR analyses are based on the GWAS. GWAS requires numerous subjects, often in multiple cohorts. Disease definition can differ among different cohorts. Third, we mostly included studies involving a predominantly European population with few individuals of other ancestries (mixed); hence, the present results may not be applicable to other racial backgrounds. Nevertheless, the present results support the results of previous epidemiology studies and promote further studies in this field.

PART III. Major depressive disorder but not bipolar disorder and schizophrenia is a causal factor for type 2 diabetes as determined by Mendelian randomization

3.1. Research Problem

To date, many epidemiologic studies have suggested the link between type 2 diabetes (T2D) and severe mental illness (SMI) including bipolar disorder (BPD), major depressive disorder (MDD), and schizophrenia (SCZ) [99]. The prevalence of T2D among individuals with SMI has been estimated to be 8% to 17% in BPD, and 16% to 25% in SCZ; further, depressed adults have a 37% increased risk of developing T2D [100-102]. The side effects of the medication used for treating SMI, unhealthy lifestyle behaviors of patients with SMI, and hypothalamic-pituitary-adrenal (HPA) axis dysregulation could contribute to the association of SMI with T2D [103, 104]. For instance, medications such as antipsychotics, antidepressants, and mood stabilizers are likely to contribute to T2D development by leading to insulin resistance or weight gain [99]. Moreover, low physical activity, poor diet, smoking, alcohol, and substance abuse in individuals with SMI might lead to T2D [105].

In contrast, T2D also affects mental health. One meta-analysis indicated that the risk of MDD increases in people with T2D [106], and a prospective population-based study showed that the prevalence of schizophrenia was significantly higher in patients with T2D than in the general population [107]. Further, a cross-sectional study reported that T2D and prediabetes may be risk factors in patients with BPD [108]. Large longitudinal studies have also demonstrated that T2D impacts mental health negatively [109]. As the results of

epidemiological studies are inconsistent, the true causality between SMI and T2D is still unclear with potential biases and confounding factors. Moreover, the difference between the onset age of T2D and SMI makes it very challenging to infer a causal relationship. In general, T2D affects middle-aged adults (after age 40) with low heritability (10–15%), while SMI usually occurs in young adulthood (in the 20–30s) with high heritability (80–85% for BPD, 80% for SCZ, and 31–42% for MDD) [110, 111]. Accordingly, we hypothesized that SMI would precede T2D, if there is a causal effect between them.

Therefore, well-designed, two-sample Mendelian randomization (MR) studies, which have widely been adopted using genetic variants such as single nucleotide polymorphisms (SNP) for instrumental variable (IV) analysis are needed to pinpoint the causal relationship between SMI and type 2 diabetes [112]. Compared to a one-sample MR, two-sample MR will not lead to inflated type 1 error rated and false-positive findings. To the best of our knowledge, a two-sample MR analysis focusing on SMI and T2D is currently lacking. For this purpose, robustness against potential confounders in MR analysis can be achieved if the three core assumptions for IV, including strong association with intermediate exposure, independence with confounders, and no direct path for the outcome are satisfied. We considered several MR approaches including an inverse-variance weighted (IVW) method and sensitivity analyses—MR-Egger, MR-Egger with a simulation extrapolation (SIMEX), weighted median approach, and MR-Pleiotropy RESidual Sum and Outlier (MR-PRESSO) method—in the case of violation of the assumptions [113].

Here, we investigate the causality between SMI and T2D using genome-wide summary statistics in a meta-analysis of a large population of European individuals through two-sample MR study.

3.2. Materials and Methods

Exposure dataset (1): summary statistics of genetic association analyses with bipolar disorder

SNPs-BPD associations were obtained from 20,352 cases and 31,358 controls who lived in 14 countries in Europe, North America, and Australia [114]. Cases were required to meet international consensus criteria (diagnostic and statistical manual of mental disorders (DSM-IV), internatinal classification of diseases (ICD)-9, or ICD-10) for a lifetime diagnosis of BPD established using structured diagnostic instruments from assessments by trained interviewers, clinician-administered checklists, or medical record reviews. A total of 9,372,253 SNPs was used for genome-wide association (GWA) analyses; among these, 16 genome-wide significant ($P < 5 \times 10^{-08}$) SNPs associated with BPD were identified after linkage disequilibrium (LD) pruning (distance < 10,000 kb or LD $r^2 < 0.001$). After removal of SNPs nominally associated with T2D ($P < 0.05$), 11 BPD-associated SNPs were available.

Exposure dataset (2): summary statistics of genetic association analyses with major depressive disorder

The summary statistics for association between SNPs-MDD were obtained through large-scale genome-wide association meta-analyses of 807,553 individuals (246,363 cases and 561,190 controls) from the UK Biobank, 23andMe, Inc., and psychiatric genomics consortium (PGC) [115]. The definition of MDD was different in each cohort. In the UK Biobank, three MDD phenotypes were used; 1) self-reported help-seeking for problems with nerves, anxiety, tension, or depression, 2) self-reported depressive symptoms, and 3) MDD identified from hospital admission records. In case of 23andMe, Inc., a self-reported clinical diagnosis of depression was used. Finally, cases were required to meet international consensus criteria (DSM-IV, ICD-9, or ICD-10) in PGC. A total of 8,098,588 SNPs was combined for meta-analysis and 50 SNPs were selected as candidates of IVs. None of them were in the same LD block ($r^2 < 0.001$) or within 10,000 kb of an established signal. We checked the pleiotropic effect of those SNPs, and eight SNPs associated with T2D ($P < 0.05$) were eliminated. Thus, 42 MDD-associated SNPs were used for our analyses.

Exposure dataset (3): summary statistics of genetic association analyses with schizophrenia

SNPs-SCZ associations were obtained from 33,640 cases and 43,456 controls from the PGC [116]. Cases with clinical diagnosis (not self-report) of SCZ or schizoaffective disorder were included in our study. Summary statistics of GWA analyses for 13,942,226 SNPs were available, and 83 variants that were genome-

wide significantly associated with schizophrenia were significant after LD pruning (distance < 10,000 kb or LD $r^2 < 0.001$). Eleven SNPs were associated with T2D ($P < 0.05$), and the remaining 72 SCZ-associated SNPs were used for our MR analyses.

Outcome dataset: summary statistics of genetic association analyses with type-2 diabetes

We obtained summary statistics for associations between SNPs-T2D from the DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium stage 1 meta-analyses with 26,676 cases and 132,532 controls [117]. T2D diagnosis was based on diagnostic fasting glucose (≥ 7 mmol/L) or HbA1c levels (≥ 6.5%), hospital discharge diagnosis, use of oral diabetes medication, or self-report. Summary statistics of GWA analyses for 12.1 million SNPs were available and were considered for our MR analyses.

Mendelian randomization analysis

All SNPs associated with BPD, MDD, or SCZ were selected separately as candidates of IVs that had genome-wide significance and were not in a LD block ($r^2 < 0.001$) or within 10000 kb of an established signal. *F*-statistics provided an indication of instrument strength and $F > 10$ indicated that the analysis was unlikely to suffer from weak instrument bias [118]. A degree of violation of the "NO Measurement Error" (NOME) assumption was quantified using I^2 statistics and $I^2 > 90$ indicated lesser dilution of the estimates in MR analysis [23]. For the detection of pleiotropic outlier SNPs, Cochran's Q-test in the inverse-variance weighted (IVW) method and Rucker Q' statistics in the MR-Egger were used [119].

We further conducted an MR-Pleiotropy RESidual Sum and Outlier (MR-PRESSO) test as an indicator of no violations of MR assumptions in the final IV sets [28]. Given that no weak instrument bias ($F > 10$) was observed and the three tests (Cochran's Q-test, Rucker Q' test, and MR-PRESSO test) indicated no directional pleiotropic bias, the IVW method was applied, which is robust when all SNPs are valid instruments [113]. Here "valid" means that the following three conditions are satisfied: (i) IVs are strongly associated with exposure, (ii) IVs independent of confounders, and (iii) IVs do not affect the outcome directly. If we assume instruments-outcome association and exposure-outcome association are denoted by β_{GY} and β_{XY}, respectively, then the IVW estimate of causal effect (i.e., effect of the exposure on the outcome) can be obtained from the inverse-variance weighted mean of ratio estimates (β_{GY}/β_{XY}). If the pleiotropy and outlier SNPs were detected from MR-PRESSO, IVW is not recommended, and we consider several sensitivity analyses to minimize bias. Weighted median method provides valid causal estimates unless more than 50% of the instruments are invalid [113]. MR-Egger method can estimate appropriate causal effects in the presence of pleiotropy effects even if all SNPs are invalid [113]. When Cochran's Q-test is rejected or both Cochran's Q and Rucker Q' tests are rejected, the MR-Egger method is recommended [113]. If the measurement error of the instruments is large ($I^2 > 90$), the method of SIMEX was applied to correct attenuation bias [23]. Furthermore, we conducted a bi-directional MR to investigate the presence of reverse-causality between SMI and type 2 diabetes. The Šidák correction for multiple comparisons was used for analysis and thus, threshold of P of $1\text{-}(1\text{-}0.05)^{\frac{1}{3}} = 0.017$ [120]. Observed power (or post-hoc power) calculations were performed using the online tool (https://sb452.shinyapps.io/power/)

[79]. The proportion of variance in the exposure explained by the genetic variants (R^2) were required for MR power analysis, and 0.23 (BPD), 0.089 (MDD), 0.24 (SCZ), and 0.196 (T2D) were used.

Table 3.1. Assumption test for instrumental variable sets

No.	Exposure	Outcome	N*	F-stat	I^2 (%)	Q-test	Q'-test	MR-PRESSO global test
1	BPD		11	32.9	96.9	0.718	0.821	0.695
2	MDD	T2D	42	37.9	97.3	0.808	0.788	0.733
3	SCZ		72	41.5	97.6	0.319	0.290	0.704
4		BPD	35	35.8	97.3	0.684	0.639	0.598
5	T2D	MDD	39	36.0	97.3	0.714	0.683	0.711
6		SCZ	43	35.8	97.2	<0.001	<0.001	<0.001

*N = Number of instruments, BPD = bipolar disorder, MDD = major depressive disorder, SCZ = schizophrenia, MR-PRESSO = MR-Pleiotropy RESidual Sum and Outlier

3.3. Results

Effect of bipolar disorder on type 2 diabetes

Eleven SNPs that genome-wide significantly associated with BPD but not T2D were used as instrument variables. All SNP-exposure and SNP-outcome effects are presented in Table 4.4. We found no evidence of weak instrument bias (*F*-statistic = 32.9), and heterogeneity and outlier pleiotropy were not observed (Q-test, *P* = 0.718; Q'-test, *P* = 0.821; MR-PRESSO global test, *P* = 0.695) (Table 3.1). The MR-Egger test also indicated no directional pleiotropic bias (intercept *P* = 0.165) and there was no violation of the NOME assumption (I^2 = 96.9%). Since all IV assumptions were satisfied, the IVW method was considered to be the most appropriate to provide unbiased estimate [113, 119]. IVW showed non-significant effect on the T2D (odds ratio (OR): 1.006, 95% CI: 0.918–1.104, *P* = 0.892). The post-hoc statistical power estimated using OR was 2.5%. Non-significant results were also obtained from other sensitivity analyses (MR-Egger, OR: 0.681, 95% CI: 0.389–1.191, *P* = 0.178; MR-Egger (SIMEX), OR: 0.995, 95% CI: 0.908–1.089, *P* = 0.917; weighted median, OR: 0.982, 95% CI: 0.868–1.111, *P* = 0.770) (Table 3.2). We checked the reverse-causal association between BPD and T2D with the 35 SNPs associated with T2D. There was no weak instrument bias (*F*-statistic = 35.8) and no violation of the NOME assumption (I^2 = 97.3%). No evidence of heterogeneity was found from the Q-test (*P* = 0.684), Q'-test (*P* = 0.639), and MR-PRESSO global test (*P* = 0.598) (Table 3.1). Non-significant reverse-causal effect was found (IVW, OR: 1.031, 95% CI: 0.971–1.095, *P* = 0.313; MR-Egger, OR: 1.051, 95% CI: 0.793–1.394, *P*=0.727; MR-Egger (SIMEX), OR: 0.993, 95% CI: 0.909–1.083, *P* = 0.867;

weighted median OR: 1.041, 95% CI: 0.956–1.134, P = 0.349) (Table 3.3). The associations of the variants with BPD and type 2 diabetes are shown in a scatter plot with four MR-fitted lines (Figure 3.1A).

Effect of major depressive disorder on type 2 diabetes

Forty-two independent SNPs that associated with MDD but not T2D were used as IVs. All SNP-exposure and SNP-outcome effects are presented in Table 3.5. Weak instrument assumption was guaranteed by F-statistic (37.9) and there was no measurement error of estimates from the MR study (I^2 = 97.3%). No evidence of heterogeneity or pleiotropy was confirmed through the Q-test (P = 0.808), Q'-test (P = 0.788), and MR-PRESSO global test (P = 0.733) (Table 3.1). Since all IV assumptions were satisfied, the IVW method was chosen and showed significant results (OR: 1.191, 95% CI: 1.036–1.372, P = 0.014). The post-hoc statistical power estimated from the ORs was 100%. Among the other sensitivity analyses, MR-Egger (SIMEX) showed a consistent significant result (OR: 1.162, 95% CI: 1.024–1.318, P = 0.025) and weighted median showed a nominally significant (P < 0.1) result (OR: 1.196, 95% CI: 0.984–1.453, P = 0.072). Non-significant result of MR-Egger may be attributable to the low power of MR-Egger (OR: 0.934, 95% CI: 0.402–2.172, P = 0.875) [26] (Table 3.2). To check the reverse-causal effect, 39 variants were used as instruments. We found that all assumptions for MR analyses were preserved (F-statistic = 36.0; Q-test, P = 0.714; Q'-test, P = 0.683; MR-PRESSO global test, P = 0.711; I^2 = 97.3%) (Table 3.1). Non-significant reverse-causal effect was found (IVW, OR: 1.002, 95% CI: 0.985–1.020, P = 0.793; MR-Egger, OR: 1.008, 95% CI: 0.949–1.070, P = 0.798; MR-Egger (SIMEX), OR: 1.002, 95% CI: 0.985–1.019, P

= 0.808; weighted median, OR: 1.007, 95% CI: 0.982–1.134, $P = 0.581$) (Table 3.3). The associations of the variants with MDD and type 2 diabetes are shown in a scatter plot with four MR-fitted lines (Figure 3.1B).

Effect of schizophrenia on type 2 diabetes

Seventy-two independent SNPs that associated with SCZ, but not T2D were found to have strong instrument strength (F-statistic = 41.5) and non-significant heterogeneity and outlier pleiotropy (Q-test, $P = 0.319$; Q'-test, $P = 0.290$; MR-PRESSO global test, $P = 0.704$) (Table 3.1). All SNP-exposure and SNP-outcome effects are presented in Table 4.6. In addition, there was no dilution bias from violation of the NOME assumption ($I^2 = 97.2\%$). The IVW method was the most powerful and showed a non-significant result (OR: 1.016, 95% CI: 0.974–1.059, $P = 0.463$). The post-hoc statistical power by estimated ORs was 10.9%. The other sensitivity analyses results showed a non-significant effect of SCZ on T2D (MR-Egger, OR: 1.012, 95% CI: 0.851–1.204, $P = 0.888$; MR-Egger (SIMEX), OR: 1.015, 95% CI: 0.981–1.060, $P = 0.511$; weighted median, OR: 1.016, 95% CI: 0.955–1.081, $P = 0.615$) (Table 3.2). To detect reserve-causal effect, forty-three SNPs were used as instruments. F-statistic showed no weak instrument bias (F-statistic = 35.8) and no violation of the NOME assumption ($I^2 = 97.2$). Heterogeneity test showed substantial evidence of outlier pleiotropy using the Q-test ($P < 0.001$), Q'-test ($P < 0.001$), and MR-PRESSO global test ($P < 0.001$) (Table 3.1). Since all three tests were rejected, the MR-PRESSO method was adopted [113] after excluding the two outlier SNPs (OR: 0.999, 95% CI: 0.991–1.009, $P = 0.987$), suggesting non-significant effect of T2D on SCZ (Table 3.3). The associations of the variants with

SCZ and T2D are shown in a scatter plot with four MR-fitted lines (Figure 3.1C).

Table 3.2. Mendelian randomization results

MR methods	parameter	N*	OR	95% CI	*P*	Power
1. Effect of BPD on T2D						
IVW	Estimate	11	1.006	[0.918, 1.104]	0.892	2.5%
MR-Egger	Intercept		1.039	[0.985, 1.095]	0.165	100%
	Slope		0.681	[0.389, 1.191]	0.178	-
MR-Egger (SIMEX)	Intercept		1.003	[1.010, 1.994]	0.540	2.1%
	Slope		0.995	[0.908, 1.089]	0.917	-
Weighted median	Estimate		0.982	[0.868, 1.111]	0.770	13.8%
MR-PRESSO	Estimate			No outlier	-	-
2. Effect of MDD on T2D						
IVW	Estimate	42	1.191	[1.036, 1.372]	0.014	100%
MR-Egger	Intercept		1.007	[0.982, 1.033]	0.566	74%
	Slope		0.934	[0.402, 2.172]	0.875	-
MR-Egger (SIMEX)	Intercept		1.004	[1.000, 1.008]	0.036	100%
	Slope		1.162	[1.024, 1.318]	0.025	-
Weighted median	Estimate		1.196	[0.984, 1.453]	0.072	100%
MR-PRESSO	Estimate			No outlier	-	-
3. Effect of SCZ on T2D						
IVW	Estimate	72	1.016	[0.974, 1.059]	0.463	10.9%
MR-Egger	Intercept		1.000	[0.987, 1.014]	0.969	6.5%
	Slope		1.012	[0.851, 1.204]	0.888	-
MR-Egger (SIMEX)	Intercept		1.001	[0.998, 1.004]	0.427	9.6%
	Slope		1.015	[0.981, 1.060]	0.511	-
Weighted median	Estimate		1.016	[0.955, 1.081]	0.615	10.9%
MR-PRESSO	Estimate			No outlier	-	-

*N = Number of instruments, BPD = bipolar disorder, MDD = major depressive disorder, SCZ = schizophrenia, MR = Mendelian randomization, IVW = inverse-variance weighted, MR-PRESSO = MR-Pleiotropy RESidual Sum and Outlier, SIMEX = simulation extrapolation, OR = odds ratio

Table 3.3. Bi-directional Mendelian randomization results

MR methods	parameter	N	OR	95% CI	*P*	Power
1. Effect of T2D on BPD						
IVW	Estimate	35	1.031	[0.971, 1.095]	0.313	18.9%
MR-Egger	Intercept		0.998	[0.974, 1.022]	0.890	52.7%
	Slope		1.051	[0.793, 1.394]	0.727	-
MR-Egger (SIMEX)	Intercept		0.997	[0.989, 1.004]	0.428	2.1%
	Slope		0.993	[0.909, 1.083]	0.867	-
Weighted median	Estimate		1.041	[0.956, 1.134]	0.349	34.3%
MR-PRESSO	Estimate			No outlier	-	-
2. Effect of T2D on MDD						
IVW	Estimate	39	1.002	[0.985, 1.020]	0.793	2.2%
MR-Egger	Intercept		0.999	[0.994, 1.005]	0.852	18.6%
	Slope		1.008	[0.949, 1.070]	0.798	-
MR-Egger (SIMEX)	Intercept		1.001	[0.999, 1.002]	0.278	2.2%
	Slope		1.002	[0.985, 1.019]	0.808	-
Weighted median	Estimate		1.007	[0.982, 1.033]	0.581	14%
MR-PRESSO	Estimate			No outlier	-	-
3. Effect of T2D on SCZ						
IVW	Estimate	43	1.006	[0.939, 1.075]	0.872	2.2%
MR-Egger	Intercept		0.997	[0.974, 1.019]	0.783	50.1%
	Slope		1.040	[0.806, 1.343]	0.758	-
MR-Egger (SIMEX)	Intercept		1.003	[0.997, 1.009]	0.246	3.3%
	Slope		1.009	[0.941, 1.082]	0.785	-
Weighted median	Estimate		0.980	[0.914, 1.050]	0.564	12.4%
MR-PRESSO	Estimate	41	0.999	[0.991, 1.009]	0.987	1%

BPD = bipolar disorder, MDD = major depressive disorder, SCZ = schizophrenia, MR = Mendelian randomization, IVW = inverse-variance weighted, MR-PRESSO = MR-Pleiotropy RESidual Sum and Outlier, SIMEX = simulation extrapolation, OR = odds ratio

Table 3.4. Instrument variables for BPD and T2D

Chr	SNP	A*	SNP-Exposure			SNP-Outcome		
			LogOR†	SE‡	*P-value*	LogOR	SE	*P-value*
2	rs2314398	C/G	1.08774	0.014	5.92×10^{-09}	-0.0009	0.013	0.940
4	rs11724116	T/C	0.90114	0.019	3.27×10^{-08}	-0.0044	0.016	0.790
5	rs329319	A/G	1.08199	0.014	1.54×10^{-08}	0.019	0.013	0.140
6	rs55648125	A/G	0.8895	0.022	4.92×10^{-08}	0.002	0.019	0.920
7	rs17150022	T/C	0.89297	0.020	2.70×10^{-08}	-0.0011	0.018	0.950
7	rs13231398	C/G	0.8863	0.022	3.36×10^{-08}	0.015	0.020	0.450
11	rs73496688	A/T	1.11483	0.019	1.05×10^{-08}	-0.0036	0.017	0.840
12	rs10744560	T/C	1.08676	0.014	2.92×10^{-09}	0.022	0.013	0.095
15	rs71395455	A/G	1.08556	0.015	1.93×10^{-08}	-0.0049	0.013	0.710
19	rs111444407	T/C	1.12367	0.018	2.40×10^{-10}	-0.018	0.017	0.300
22	rs138321	A/G	1.08253	0.014	4.69×10^{-09}	-0.0043	0.012	0.720

*A: risk/reference allele, †LogOR: log odds ratio, ‡SE: standard error

Table 3.5. Instrument variables for MDD and T2D

Chr	SNP	A*	SNP-Exposure			SNP-Outcome		
			LogOR†	SE‡	*P-value*	LogOR	SE	*P-value*
1	rs4141983	T/C	0.026	0.005	9.69×10^{-09}	0.014	0.013	0.280
1	rs354155	C/G	-0.045	0.008	1.75×10^{-09}	-0.009	0.020	0.640
1	rs7551758	T/G	-0.028	0.004	5.11×10^{-11}	0.013	0.012	0.270
1	rs6656912	T/C	-0.025	0.004	6.50×10^{-09}	-0.020	0.012	0.100
1	rs10913112	T/C	-0.026	0.005	4.53×10^{-09}	0.002	0.013	0.880
1	rs17641524	T/C	-0.030	0.005	1.50×10^{-08}	0.019	0.015	0.180
2	rs2111592	A/G	0.026	0.005	1.35×10^{-08}	-0.005	0.013	0.720
2	rs72948506	A/G	0.027	0.005	1.72×10^{-08}	0.024	0.013	0.072
3	rs9831648	T/G	-0.029	0.005	1.59×10^{-08}	0.002	0.015	0.900
3	rs843812	A/G	0.025	0.004	1.41×10^{-08}	0.014	0.012	0.240
3	rs76954012	A/T	0.041	0.007	2.41×10^{-08}	0.022	0.021	0.300
3	rs66511648	T/C	-0.030	0.005	6.03×10^{-10}	0.001	0.014	0.970
3	rs12631196	A/G	0.024	0.004	3.28×10^{-08}	0.000	0.012	0.990
5	rs30266	A/G	0.037	0.005	1.43×10^{-15}	0.016	0.013	0.230
5	rs7725715	A/G	0.029	0.004	1.61×10^{-11}	-0.008	0.012	0.500
6	rs2232423	A/G	0.062	0.007	1.14×10^{-18}	0.002	0.023	0.940
6	rs2214123	A/G	0.026	0.005	8.56×10^{-09}	0.015	0.013	0.230
6	rs9364755	A/G	-0.028	0.005	3.49×10^{-08}	0.002	0.016	0.900
7	rs10235664	T/C	0.027	0.005	4.68×10^{-08}	0.012	0.014	0.380
7	rs3807865	A/G	0.031	0.004	1.09×10^{-12}	0.021	0.013	0.093
7	rs59082935	T/C	0.036	0.007	3.07×10^{-08}	0.005	0.019	0.800
7	rs2247523	C/G	-0.024	0.004	1.71×10^{-08}	-0.023	0.012	0.052
7	rs4730387	A/T	0.024	0.004	4.12×10^{-08}	0.012	0.012	0.310
7	rs150346963	T/C	0.028	0.004	1.16×10^{-10}	0.018	0.013	0.140
9	rs1931388	A/G	0.030	0.004	1.68×10^{-11}	0.003	0.012	0.830
9	rs59283172	A/G	-0.039	0.007	2.41×10^{-08}	-0.002	0.020	0.910
9	rs2418449	T/C	0.028	0.005	4.25×10^{-09}	0.015	0.013	0.270
10	rs1021363	A/G	0.030	0.005	2.29×10^{-11}	0.004	0.013	0.750
11	rs198457	T/C	-0.032	0.006	1.90×10^{-08}	0.024	0.016	0.130
11	rs10501696	A/G	0.030	0.004	2.89×10^{-11}	0.017	0.013	0.200
12	rs61914045	A/G	0.031	0.005	7.96×10^{-09}	-0.009	0.015	0.560
13	rs9529218	T/C	-0.034	0.005	2.23×10^{-10}	-0.021	0.015	0.170
13	rs9536381	T/C	0.026	0.005	2.62×10^{-08}	0.013	0.013	0.320
13	rs508502	T/C	-0.026	0.005	3.56×10^{-08}	0.014	0.014	0.310
14	rs1950829	A/G	0.030	0.004	4.74×10^{-12}	0.005	0.012	0.660
14	rs754287	A/T	-0.029	0.005	1.31×10^{-10}	0.008	0.013	0.530
15	rs28541419	C/G	0.029	0.005	1.76×10^{-08}	-0.016	0.016	0.300
16	rs12919291	C/G	0.033	0.006	3.09×10^{-09}	0.009	0.016	0.570
18	rs4799949	T/C	-0.029	0.005	1.40×10^{-10}	-0.009	0.013	0.510
18	rs1367635	T/C	-0.025	0.004	4.35×10^{-09}	0.008	0.012	0.530
18	rs12967143	CG	-0.035	0.005	2.53×10^{-13}	-0.006	0.013	0.640
20	rs13037326	T/C	0.031	0.005	2.40×10^{-10}	0.002	0.014	0.900

*A: risk/reference allele, †LogOR: log odds ratio, ‡SE: standard error

Table 3.6. Instrument variables for SCZ and T2D

Chr	SNP	Allele*	SNP-Exposure			SNP-Outcome		
			LogOR†	SE‡	*P-value*	LogOR†	SE‡	*P-value*
1	rs301798	A/G	0.937	0.012	2.04×10^{-08}	-0.007	0.013	0.600
1	rs533123	A/G	1.081	0.014	3.64×10^{-08}	0.002	0.016	0.900
1	rs6694545	A/G	1.082	0.013	6.06×10^{-08}	0.007	0.015	0.650
1	rs11210892	A/G	0.935	0.012	4.13×10^{-08}	0.003	0.013	0.850
1	rs10890030	T/C	0.936	0.011	1.22×10^{-09}	-0.012	0.012	0.330
1	rs2802535	T/C	1.125	0.014	1.61×10^{-17}	-0.006	0.015	0.690
1	rs2319280	A/C	0.913	0.015	9.61×10^{-10}	-0.029	0.016	0.075
1	rs12093576	T/C	1.065	0.011	1.31×10^{-08}	-0.010	0.013	0.430
2	rs12712510	T/C	1.065	0.011	2.38×10^{-08}	0.022	0.012	0.075
2	rs12474906	A/C	1.085	0.014	7.98×10^{-09}	-0.011	0.015	0.480
2	rs11682175	T/C	0.929	0.011	4.61×10^{-11}	-0.023	0.012	0.056
2	rs16825349	A/G	0.923	0.014	1.84×10^{-08}	0.013	0.016	0.420
2	rs4340536	C/G	0.942	0.011	3.57×10^{-08}	0.013	0.012	0.300
2	rs10196799	A/T	1.077	0.011	1.12×10^{-11}	0.007	0.012	0.550
2	rs55775495	T/C	0.934	0.011	1.91×10^{-09}	-0.006	0.013	0.630
2	rs2949006	T/G	1.110	0.014	3.45×10^{-14}	0.002	0.015	0.890
2	rs4144795	C/G	1.082	0.011	3.12×10^{-12}	-0.019	0.013	0.150
3	rs17194490	T/G	1.104	0.015	1.69×10^{-11}	-0.012	0.017	0.470
3	rs832187	T/C	0.933	0.011	7.33×10^{-10}	0.006	0.013	0.650
3	rs62244881	T/C	1.097	0.016	1.29×10^{-08}	0.022	0.018	0.210
3	rs12163529	A/G	0.932	0.011	1.59×10^{-10}	0.000	0.012	0.980
3	rs13071962	A/G	0.922	0.014	1.82×10^{-09}	0.015	0.015	0.330
4	rs215412	A/G	1.070	0.012	3.56×10^{-09}	0.017	0.013	0.180
4	rs13107325	T/C	1.166	0.021	3.85×10^{-13}	0.017	0.029	0.570
4	rs7683893	T/C	0.941	0.011	3.40×10^{-08}	0.019	0.013	0.140
5	rs4391122	A/G	0.925	0.011	5.70×10^{-13}	-0.017	0.012	0.160
5	rs301714	C/G	1.162	0.026	6.59×10^{-09}	0.026	0.027	0.330
5	rs3849046	T/C	1.064	0.011	1.30×10^{-08}	0.008	0.012	0.510
5	rs3112532	A/G	0.933	0.011	1.57×10^{-09}	0.008	0.013	0.530
5	rs11740474	A/T	0.939	0.011	1.50×10^{-08}	0.024	0.012	0.052
6	rs1233578	A/G	1.208	0.016	1.48×10^{-31}	-0.015	0.021	0.470
6	rs1339227	T/C	0.938	0.011	2.64×10^{-08}	0.010	0.013	0.410
6	rs217289	A/G	0.936	0.011	1.58×10^{-09}	-0.003	0.012	0.800
6	rs117074560	T/C	0.847	0.027	5.46×10^{-10}	0.002	0.032	0.960
7	rs12532143	T/C	1.084	0.012	2.33×10^{-12}	-0.018	0.013	0.170

*A: risk/reference allele, †LogOR: log odds ratio, ‡SE: standard error

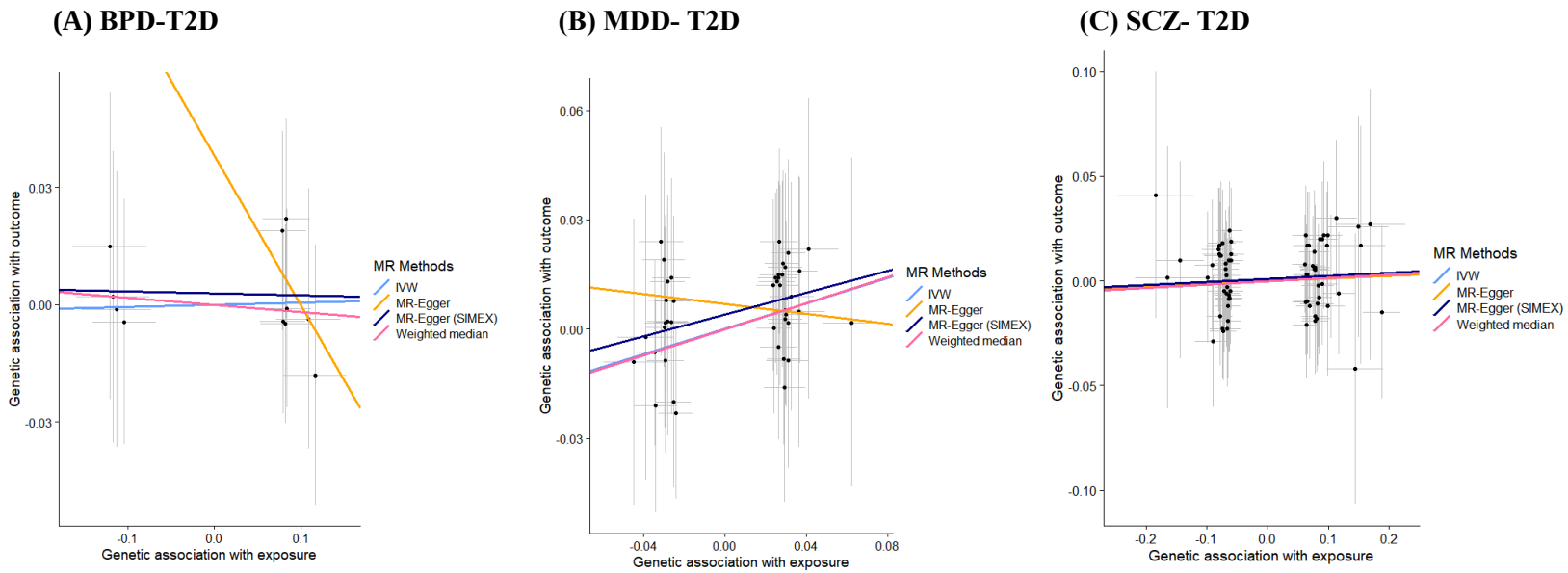


Figure 3.1. Scatter plots showing the associations of the genetic variants with psychiatric disorders and type 2 diabetes for different methods of Mendelian randomization. **(A)** The associations of the variants with BPD and type 2 diabetes. **(B)** The associations of the variants with MDD and type 2 diabetes. **(C)** The associations of the variants with SCZ and type 2 diabetes.
Abbreviations: BPD = bipolar disorder, MDD = major depressive disorder, SCZ = schizophrenia, MR = Mendelian randomization, IVW = inverse-variance weighted, SIMEX = simulation extrapolation

3.4. Discussion

In the present study, two-sample MR results provided solid evidence in support of the hypothesis that MDD increases the risk of T2D, whereas BPD and SCZ were not identified as risk factors for type 2 diabetes. Bi-directional MR study also confirmed that there was no reverse-causality between SMI and T2D, and the asymmetry in the effects of the instruments on type 2 diabetes and MDD supports the fact that MDD is one of the causal factors that influences type 2 diabetes. However, causal effects of MDD on T2D development have been demonstrated to be controversial in observational studies. One systematic review demonstrated that MDD is associated with a 60% increased risk of T2D, while the evidence is also compatible with the high prevalence rates of MDD among individuals with type 2 diabetes [121]. A large meta-analysis showed that T2D is associated with only a modestly increased risk of MDD [122]. MDD is difficult to detect in older adults which may partially explain why this association was so modest [123].

Our finding, the causal role of MDD in increased risk of T2D, could be explained by the pathophysiological mechanisms underlying the two diseases. Two major molecular mechanisms have been suggested to explain the causal pathway between them. First, the HPA axis, a central stress response system is commonly activated in MDD patients suffering from emotional stressors, leading to a rise in the levels of glucocorticoids, primarily cortisols [124]. High cortisol level induces and aggravates insulin resistance in a vicious cycle, such as increased β-cell function and increased insulin release to glucose challenge by exacerbating progression to insulin resistance [125]. Second, the sympathetic nervous system (SNS) activity is also

elevated in MDD [126]. The SNS axis interacts complexly with the HPA axis to maintain homeostasis during stress, resulting in an increased release of cortisol and other glucocorticoids, catecholamines, growth hormone, and glucagon. Indeed, the catecholamines have marked metabolic effects, particularly on glucose metabolism [127].

However, our findings are inconsistent with some observational study suggesting a causal role of BPD and SCZ in the risk of T2D and that T2D predicts the development of MDD [101]. Such associations may have been driven by residual confounding, because many aspects of the relationship between SMI and T2D are yet to be examined in a controlled manner, and there are many suggestive evidences that can act as confounders. First, sedentary lifestyle and low physical activity, which have been demonstrated to be strongly associated with SMI, may play a role as a potential confounder [99]. A large meta-analysis of general population studies reported that sedentary behavior is independently associated with an increased risk of T2D [128]. In addition, side effects of medication could also be another important potential confounder. A systematic review of cross-sectional and prospective studies of psychotropic medications and physical diseases indicated that the use of antipsychotics, antidepressants, and mood stabilizers can contribute to an increased BMI which is a major risk factor for T2D [129]. Especially, antipsychotics, such as clozapine and olanzapine; antidepressants, such as paroxetine; and mood stabilizers, such as lithium and valproate have been associated with increasing obesity. Further, the prevalence of smoking behaviors (daily smoking) was the highest in SCZ, followed by BPD and MDD compared with the general population and the association with smoking is very strong in SCZ and BPD, while it is less strong in

MDD [130]. The evidence that nicotine addiction begins before any of these SMIs develop suggests that there are shared genes associated with nicotine addiction and SMI [131].

MR studies on the association between SMI and T2D are scarce, with no studies on BPD with T2D. To investigate the potential causal relationship of T2D with MDD, MR analysis was performed with a large longitudinal cohort from 2011 to 2013 in China [42]. Genetic risk scores for T2D were chosen as the instruments and two-stage multiple regression was used for statistical analysis. The results provided evidence of a potential causal effect of T2D on MDD, which is the opposite of our results. We thought that there is a finite-sample bias in the existing research, because in a one-sample setting, the fitted values from the first-stage regression are correlated with the outcome in finite samples even in the absence of a causal effect [43]. Regarding the MR studies of SCZ and T2D, two-sample MR was performed using the IVW and MR-Egger methods in Europeans, East Asians, and trans-ancestry groups [44]. No evidence of a causal role of T2D for SCZ was observed in any of the analyses, which is consistent with our findings; however, they did not perform bi-directional analysis for causal effect of SCZ on T2D. Unlike epidemiological studies, the previous and the present MR studies could not consider multi-episode status of disease, which may have led to non-significant results of SCZ and BPD. This could be because multi-episode (versus first-episode) persons with SMI were significantly more likely to have T2D than matched controls in the meta-analysis of observational studies [99].

Several limitations of our study need to be acknowledged. First, there are different clinical subtypes of MDD (melancholic, psychotic, atypical, or

undifferentiated) and bipolar disorder (type 1 or 2) and mood states (manic, depressive, mixed, or euthymic); however, a large category of diseases was analyzed without distinction. Second, although we conducted bi-directional MR studies, the sample size of GWA studies for instruments and BPD/SCZ (less than 100,000) was relatively small, which could lead to low power of the analysis. Third, we only included European population; hence, it is difficult to apply the same clinical interpretation to other races. Nevertheless, the present study has implications in that the well-designed, two-sample MR study for SMI and T2D produced less biased results than those of the existing epidemiological studies or one-sample MR study. In addition, the present study clearly showed the causality of MDD on T2D, which is supported by previously reported underlying biological mechanisms. Therefore, it is imperative to consider screening for diabetes and metabolic abnormalities in patients with MDD or probable MDD.

Conclusions

The first goal of this research is to investigate the impact of genetic variants on longitudinal change of fasting glucose. Our findings provide evidence in support of that the genetic effect on longitudinal change of fasting glucose may be weak, which in line with results from European study. Since a little of genetic risk factors for longitudinal deterioration of fasting glucose has been explained and not been widely studied, further replication studies are required to validate our findings. The second final goal is to identify causal effect of T2D-related traits on cancers/vascular disease and severe mental illness (SMI) on T2D by using Mendelian randomization (MR) study. Even if some traits have been studied previously in epidemiology study, it is worth noting all the results from our MR analyses. Because our results were not affected by the observational confounded association, potential biases and reverse causation. To know the direction of any causal relation between health effects might give an opportunity for preventing patients with abnormal fasting glucose, 2h plasma glucose and HbAlc from severe disease or patients with SMI from T2D.

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Abstract in Korean

연구배경: 유전체 분석 기술의 급속한 발달로 유전자형 데이터가 폭발적으로 증가함에 따라, 제2형 당뇨병에 대한 전장 유전체 연관 분석(GWAS) 은 당뇨병과 관련된 400 개 이상의 유전자 좌(loci)와 공복 혈당 및 공복 인슐린 수준에 대해 약 120 개의 유전자 좌를 확인하였다. 그러나 공복 혈당의 종단적 변화에 영향을 주는 유전인자에 대한 연구는 현재까지 보고된 바가 많지 않다. 한편 여러 임상연구들에서는 제 2형 당뇨가 다른 많은 질병들과 상관관계가 있다는 사실을 밝혀왔다. 그러나 이러한 역학연구들은 여러가지 편향(bias) 및 교란변수 그리고 역인과관계에 취약한 단점을 가지고 있다.

연구목적: 이 연구의 목적은 (1) 공복 혈당의 종단적 변화와 관련된 유전 적 변이를 확인하고 (2) 멘델 무작위 분석법(MR)을 통해 제 2형 당뇨의 관련 형질인 공복 혈당, 식후 2시간 혈당, 당화혈색소 (HbA1c)에 의해 영향을 받을 수 있는 암 및 혈관질환을 확인하고 (3) 정신병과 제 2형 당뇨병 사이의 인과관계를 규명하는 것이다.

연구방법: 공복 혈당의 종단적 변화에 대한 유전 변이를 조사하기 위해, 초기 검사에서 당뇨병이 없는 한국인들로 구성된 두 개의 전향적 코호트를 이용하였다. 선형 혼합 모형으로 분석을 수행하였고 두 코호트의 결과를 METAL 소프트웨어를 사용하여 메타분석을 하였다. 제2형 당뇨와 관련된 지표와 다양한 질병 및 건강 지표들의 인과관계를 규명하기 위해서는, 당뇨를 갖고 있지 않은 133,010명의 유럽인들의 대규모 GWAS 메타분석을 통해 얻어진 요약 통계량을 활용하였다. 이는 공복혈당 및 인슐린 관련 형질 컨소시엄(MAGIC)에서 제공하는 데이터이다. 또한 MR-BASE 플랫폼을 통해 총 70개의 질병 및 건강지표들과 이와 관련된 GWAS 결과 값을 얻었다. 이 플랫폼은 1,673개의 GWAS를 통해 얻어진 약 110 억 개의 단일 핵산염기 다형현상 (SNP)의 결과 값을 가지고 있다. 여기에서 얻어진 도구 변수들은 Q-통계량, Q’-통계량, MR-PRESSO(MR-Pleiotropy RESidual Sum and Outlier) 테스트를 통해

타당성을 확인하였다. 그런 다음 도구변수들의 특성에 맞는 MR 분석 기법이 적용되었고, 다음과 같은 여러 MR방법이 고려되었다: (1) Inverse variance weighted (2) MR−Egger (3) MR−Egger with SIMEX (4) MR−PRESSO (5)weighted median method.

연구결과 및 결론: *PLCE1 (phospholipase C epsilon 1)*의 인트론에 위치하는 SNP인 rs11187850는 공복 혈당의 종단적 변화에 유의한 연관성을 보였으며 ($P = 4.85\times10^{-08}$), 세 가지 SNP은 연관성을 암시하는 결과를 보였다. 세 가지의 SNP은 각각 *NUDT3 (nucleoside diphosphate linked moiety X−type motif 3)*의 근처에 있는 rs10947494 ($P = 3.64\times10^{-06}$), *MIR6085 (MicroRNA 6085)*의 근처에 있는 rs2414772 ($P = 6.30\times10^{-06}$), *USB1 (U6 snRNA biogenesis phosphodiesterase 1)*의 근처에 있는 rs16959641 ($P = 2.64\times10^{-06}$) 이었다. MR 분석에서는 관상 동백 질환이 높은 공복 혈당 값에 의해 발병 확률이 높아지는 영향을 받았고 또한, LDL 콜레스테롤은 HbAlc과 양의 인과 관계가 있음을 확인하였다. 더불어, 우울증이 당뇨의 유의한 위험인자라는 것을 보였고, 반면 조울증과 조현병에서는 인과관계가 없음을 확인하였다. 위의 결과들은 기존의 역학 연구의 결과들을 뒷받침하며 더욱 강력한 근거를 제시하기도 하였고, 혹은 역학 연구와 반대되는 결과를 제시함으로써 새로운 시각을 제시하기도 하였다.