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Diabetes and Risk of Arterial Stiffness: A Mendelian Randomization Analysis

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We aimed to explore the causal association between type 2 diabetes (T2D) and increased arterial stiffness. We performed a Mendelian randomization (MR) analysis in 11,385 participants from a well-defined community study in Shanghai during 2011–2013. We genotyped 34 T2D-associated common variants identified in East Asians and created a genetic risk score (GRS). We assessed arterial stiffness noninvasively with the measurement of brachial-ankle pulse wave velocity (baPWV). We used the instrumental variable (IV) estimator to qualify the causal relationship between T2D and increased arterial stiffness. We found each 1-SD increase in T2D_GRS was associated with 6% higher risk in increased arterial stiffness (95% CI 1.01, 1.12), after adjustment of other metabolic confounders. Using T2D_GRS as the IV, we demonstrated a causal relationship between T2D and arterial stiffening (odds ratio 1.24, 95% CI 1.06, 1.47; *P* = 0.008). When categorizing the genetic loci according to their effect on insulin secretion or resistance, we found genetically determined decrease in insulin secretion was associated with increase in baPWV (β_{IV} = 122.3 cm/s, 95% CI 41.9, 204.6; *P* = 0.0005). In conclusion, our results provide evidence supporting a causal association between T2D and increased arterial stiffness in a Chinese population.

Atherosclerotic disease is the major complication of type 2 diabetes (T2D) and the leading cause of high mortality in T2D (1,2). Atherosclerosis leads to the degeneration of arterial elasticity (3). In T2D patients, increased arterial stiffness is a strong risk factor for cardiovascular outcomes and early mortality (4). Growing epidemiology evidence has shown that T2D is associated with increased arterial stiffness (5–8). However, randomized controlled trials (RCTs) assessing the effect of glucose lowering on cardiovascular outcomes have yielded mixed results (9–12). Because conventional epidemiological studies are subject to a variety of bias, such as confounding or reverse causation, systematical investigations of causal relation between T2D and arterial stiffening are needed. Recently, the Mendelian randomization (MR) analysis has been widely used for assessing causality in the cardiovascular risk epidemiological studies using genetic variants as the instrumental variable (IV) (13–16). Genetic alleles are allocated randomly during gamete formation, and the common variants are inherited independent of potential confounding factors (17). Therefore, the use of the genetic factors as the IV is regarded as independent of confounders in the effect on the intermediate phenotypes–outcome relationship.

Pulse wave velocity (PWV) is a noninvasive measurement of arterial stiffness and an independent predictor of cardiovascular diseases (18). Brachial-ankle PWV (baPWV) has been widely used for screening increased arterial stiffness in a large population (19), and higher baPWV value has been related to atherosclerotic vascular damage and cardiovascular risk (20).

In a large community-based sample of Chinese participants, we performed an MR analysis to explore the causal

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association between T2D and increased arterial stiffness defined as elevated baPWV. We created a T2D genetic risk score (GRS) to represent the overall genetic susceptibility (21,22) and tested the causal relation between genetically determined T2D and risk of increased arterial stiffness using the MR approach. We also analyzed the genetic variations according to their roles in insulin secretion (IS) and insulin resistance (IR).

RESEARCH DESIGN AND METHODS

Study Subjects

This study was a part of an ongoing investigation of the Risk Evaluation of cAncers in Chinese diabeTic Individuals: a lONgitudinal (REACTION) study, which is a large, nationwide, prospective study involving 259,657 community-dwelling adults, aged 40 years or older (23,24). Briefly, the participants of the current study were recruited from two nearby communities in Baoshan district of Shanghai, China, in 2011 and in 2013. A standard questionnaire was used to collect information about lifestyle factors, disease, and medical history. Anthropometric measurements and 75-g oral glucose tolerances tests (OGTTs) were performed. Blood and urine samples were collected.

There were 11,935 participants (average age 63.5 years and 35.6% men) recruited in the study, in which genotype information was available in 11,837 participants (99.2%). We excluded the participants who were missing information on baPWV (n = 216) or who had more than two single nucleotide polymorphism (SNP) genotypes missing (n = 236); thus, 11,385 participants were involved in the final analysis. The institutional review board of Ruijin Hospital of Shanghai Jiao Tong University School of Medicine approved the study protocol. Each participant gave written informed consent.

Anthropometric and Laboratory Measurements

We used a standard questionnaire to collect the social demographic information, history of chronic diseases, use of medications, and lifestyle factors, such as habits of smoking, drinking and physical activity, etc. The current smoking or drinking status were defined as "yes" if the subject smoked at least one cigarette or consumed alcohol at least once a week in the past 6 months. Physical activity at leisure time was assessed using the short form of the International Physical Activity Questionnaire (IPAQ) (25) by adding questions on duration of mild/moderate/vigorous activities per day. Body height and weight were measured by trained investigators. BMI was calculated as body weight in kilograms divided by height squared in meters (kg/m^2). Systolic and diastolic blood pressures (SBP and DBP) were measured in triplicate on the same day after at least 10-min rest using an automated electronic device (OMRON Model HEM-752 FUZZY, Omron Co., Dalian, China), and the average value of the three measurements was used for analysis.

All participants underwent OGTT and fasting and 2-h blood samples were obtained to measure the biomarkers at the same laboratory. Fasting and OGTT 2-h plasma glucose (FPG and OGTT-2h PG) were measured using the hexokinase method on a clinical chemistry diagnostic system (*c*16000, Abbott Diagnostics, Otawara-shi, Japan). Fasting serum total cholesterol (TC), triglycerides (TG), HDL cholesterol, and LDL cholesterol were measured using the clinical chemistry diagnostic system (*c*16000, Abbott Diagnostics, Otawara-shi, Japan). Serum fasting insulin was measured using the immunoassay diagnostic system (*i*2000, Abbott Diagnostics, Dallas, TX). IR index (HOMA-IR) was calculated as fasting insulin (μIU/mL) × FPG (mmol/L)/22.5. HOMA of β-cell function or IS (HOMA-β) was calculated using the formula: HOMA-β = [20 * fasting insulin (μIU/mL)]/[FPG (mmol/L) − 3.5].

Measurement of PWV

baPWV indicates brachial-to-ankle PWV. It was determined by a fully automatic arteriosclerosis diagnosis device (Colin VP-1000, model BP-203RPE II, form PWV/ABI) with the participants in the supine position after resting for 10–15 min. To determine the baPWV, pulse waves were measured simultaneously with cuffs placed on the right upper arm and the right ankle. The difference in the times of the start of the pulse waves was corrected for distance to obtain the baPWV (26).

Definitions

According to the 1999 World Health Organization diagnostic criteria, T2D was defined as FPG ≥7.0 mmol/L or OGTT-2h PG ≥11.1 mmol/L or self-reported physician-diagnosed diabetes and use of antidiabetes agents. The blood pressure greater than 140 mmHg in SBP or 90 mmHg in DBP or use of antihypertensive medications was diagnosed as hypertension. The fourth quartile of baPWV (1,902 cm/s) in the current study was defined as increased arterial stiffness.

Genotyping and Quality Control

Blood white cells were collected for DNA extractions using commercial blood genomic DNA extraction kit (OSR-M102-T1, TIANGEN BIOTECH Co., Ltd., Beijing, China) on an automated nucleic acid extraction instrument (OSE-M48, TIANGEN BIOTECH Co., Ltd., Beijing, China) according to the manufacturer's standard protocol. Specific assays were designed using the MassARRAY assay design software package (v3.1) (https://www.agenacx.com/Home). Mass determination was carried out with the MALDI-TOF mass spectrometer and data acquisition was performed using MassARRAY Typer 4.0 software (Sequenom, CapitalBio Corp., Beijing, China). The minimum call rate was 98.7%. The concordance rate was more than 99% based on 100 duplicates genotyping (27).

Genetic Loci Selection and GRS Construction

On considering the ethnicity specificity in genetic background, we selected the SNPs that have been discovered in Europeans and successfully replicated in East Asians (28,29) or those that were identified and validated in a

meta-analysis including genome-wide association studies (GWAS) from East Asians (29). We presented the full list of the SNPs in Supplementary Table 1. They all reached a genome-wide significance level ($P < 5 \times 10^{-8}$) and not in linkage disequilibrium ($r^2 = 0$, except it was 0.055 between rs10906115 and rs12779790 in *CDC123/CAMK1D*). The characteristics of the individual SNP in the T2D_GRS and the association of each SNP with T2D are shown in Supplementary Table 1. For the GRS construction, we assumed the additive genetic model (17) for each SNP. The weighted GRS was the sum of the number of risk alleles weighted by the effect size (the natural log of the odds ratios [ORs]) for risk of T2D summarized in the literature (30). We excluded the participants who were missing more than two SNPs (n = 236); thus, 11,385 participants were involved in the final main analysis. With those who were missing one or two SNPs, we assigned them the average genetic score. Using these 34 SNPs, we constructed a weighted T2D_GRS (mean ± SD 34.52 ± 3.89) and an unweighted T2D_GRS (35.88 ± 3.61). All the results in the current study were for the weighted score and the unweighted score was used in the sensitivity analysis.

We further categorized the T2D SNPs into IS- or IR-related loci (Supplementary Table 2). Among the 34 T2D SNPs, 16 were significantly associated with IS indicated as Log_{10}-HOMA-β and 6 were associated with IR indicated as Log_{10}-HOMA-IR (nominal $P < 0.05$). Then, we created a weighted IS_GRS ranging from 6.48 to 25.60 and a weighted IR_GRS ranging from 3.20 to 12.00, based on weighting each allele with the effect size (β) on association with Log_{10}-HOMA-β or Log_{10}-HOMA-IR, respectively.

Statistical Analysis

SAS version 9.3 (SAS Institute, Cary, NC) was used for database management and statistical analysis. Serum TG, HOMA-IR, and HOMA-β were normalized by logarithmic transformation because of skewed distributions before statistical analysis. Categorical variables were shown in proportions. Linear regression analysis was used to test for trend across the T2D_GRS quartiles for continuous variables, and the Cochran-Armitage trend χ^2 test was used for categorical variables. Multivariable linear regression models were fitted to evaluate the association of T2D_, IR_, and IS_GRSs with baPWV, respectively. Multivariate logistic regression models were used to assess the risk of increased arterial stiffness related to the presence of T2D and T2D_GRS. Model 1 was adjusted for age (years), sex, and BMI (kg/m^2); model 2 was further adjusted for current smoking (yes or no), current drinking (yes or no), physical activity (mild, moderate, or vigorous), hypertension (yes or no), and serum TC (mmol/L), HDL cholesterol (mmol/L), LDL cholesterol (mmol/L), and TG (mmol/L). Models 3 and 4 were based on model 2 and further adjusted for FPG and OGTT-2h PG levels or the T2D status (yes or no), respectively. Statistical significance was set to a two-sided P value of less than 0.05.

In the MR analysis, we used the weighted T2D_GRS as the IV estimators to measure the strength of the causal relationship between T2D and risk of increased arterial stiffness. The IV estimate of causal OR was derived using the Wald-type estimator (31) and then exponentiated to express as an OR. The computational formula was $OR_{IV} = \exp(\text{Ln}(OR_{\text{GRS-arterial stiffness}})/\text{Ln}(OR_{\text{GRS-T2D}}))$. For the causal association of increase in baPWV in relation to IS or IR, the IV estimate (β_{IV}) was calculated as a ratio between the two regression coefficients: $\beta_{IV} = \beta_{GRS\_baPWV}/\beta_{GRS\_HOMA\ (\beta\ or\ IR)}$. For increased arterial stiffness risk, we tested the null hypothesis of no difference between the IV estimators and the conventional regression-based estimators for the effect of T2D, IS, or IR via a classical z-test. The IV estimates were adjusted for similar adjustments as above, including age, sex, BMI, current smoking, current drinking, physical activity, hypertension, and lipids.

We assessed the potential pleiotropic effects of each individual SNP and the T2D_GRS as well. We conducted the sensitivity analysis using the unweighted GRS, the GRS excluding the SNPs that were associated with other T2D-related metabolic traits, or the SNP with the strongest association with increased arterial stiffness as the IVs. In addition, we included those participants who were missing more than two SNPs (n = 236) as a missing indicator in the sensitivity analysis.

RESULTS

Genetic Loci and T2D_GRS

The associations of the each individual SNP with risk of increased arterial stiffness are summarized in Supplementary Fig. 1. Most of the individual SNPs did not show significant association with increased arterial stiffness, except the *SPRY2* rs1359790 (Nominal P = 0.01). A total of 16 SNPs were found to be nominally related to sex distribution, BMI, SBP, DBP, TC, or Log-TG ($P < 0.05$, Supplementary Table 3).

The weighted T2D_GRS ranged from 21.0 to 49.4 (Supplementary Fig. 2). As expected, the T2D_GRS was associated with an increase in glucose metabolism–related traits, such as FPG, OGTT-2h PG, and percentage of T2D patients and a decrease in HOMA-β (all $P < 0.05$, Table 1 and Supplementary Table 4). In addition, the T2D_GRS was nominally associated with sex distribution, BMI, and SBP (all $P \leq 0.02$, Supplementary Table 4). The level of baPWV and the percentage of patients with increased arterial stiffness were increased across the T2D_GRS quartiles (Table 1).

Associations of T2D_GRS and T2D With Increased Arterial Stiffness

As shown in Table 2, per SD (3.89 points) increase in T2D_GRS was associated with a 6% increased risk of increased arterial stiffness after adjustment for age, sex, and BMI (95% CI 1.01, 1.11; P = 0.02) (model 1). Further adjustment for smoking, drinking status, physical activity, hypertension, and serum lipids did not appreciably change the results (OR 1.06 [95% CI 1.01, 1.12]; P = 0.01) (model 2). The categorical analysis showed similar

Table 1—Characteristics of study participants according to the weighted T2D_GRS (*n* = 11,385)

	T2D_GRS				
	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P* for trend
n	2,842	2,851	2,846	2,846	—
T2D_GRS	29.57 (1.92)	33.26 (0.74)	35.77 (0.75)	39.46 (1.91)	< 0.0001
Age, years	63.2 (9.8)	63.4 (9.8)	63.3 (9.8)	63.1 (10.0)	0.72
Male, *n* (%)	975 (34.3)	973 (34.1)	1,037 (36.4)	1,058 (37.2)	0.03
BMI, kg/m^2	25.4 (3.6)	25.4 (3.7)	25.2 (3.6)	25.1 (3.5)	0.0002
Current smoking, *n* (%)	388 (14.5)	390 (14.5)	402 (14.9)	434 (16.0)	0.10
Current drinking, *n* (%)	240 (9.0)	241 (9.0)	264 (9.9)	276 (10.3)	0.05
Physical activity, mild/moderate/vigorous (%)	77.6/16.4/6.0	76.8/17.3/6.0	76.3/17.6/6.2	78.3/16.4/5.3	0.58
SBP, mmHg	136 (20)	137 (20)	137 (20)	137 (21)	0.36
DBP, mmHg	78 (10)	77 (10)	77 (10)	77 (10)	0.11
FPG, mmol/L	5.78 (1.56)	5.96 (1.61)	6.06 (1.77)	6.19 (1.85)	< 0.0001
OGTT-2h PG, mmol/L	8.33 (3.68)	8.81 (4.05)	9.02 (4.21)	9.36 (4.31)	< 0.0001
HOMA-IR	1.6 (1.1, 2.4)	1.6 (1.1, 2.4)	1.6 (1.1, 2.3)	1.6 (1.1, 2.3)	0.94
HOMA-β	65.1 (44.9, 91.8)	60.8 (42.4, 85.1)	58.6 (39.7, 82.1)	54.4 (37.5, 77.4)	< 0.0001
Serum TG, mmol/L	1.30 (0.94, 1.82)	1.27 (0.93, 1.83)	1.27 (0.94, 1.80)	1.27 (0.91, 1.84)	0.21
Serum TC, mmol/L	4.93 (1.19)	4.94 (1.16)	4.94 (1.18)	4.95 (1.19)	0.69
Hypertension, *n* (%)	1,661 (58.5)	1,630 (57.2)	1,634 (57.4)	1,622 (57.0)	0.32
Antihypertensive treatment, *n* (%)	632 (22.2)	624 (21.9)	635 (22.3)	593 (20.8)	0.28
T2D, *n* (%)	525 (18.5)	682 (23.9)	745 (26.2)	883 (31.0)	< 0.0001
T2D therapy, *n* (%)	236 (8.3)	348 (12.2)	355 (12.5)	507 (17.8)	< 0.0001
baPWV, cm/s	1,682 (382)	1,690 (387)	1,700 (383)	1,708 (387)	0.006
Increased arterial stiffness, *n* (%)	690 (24.3)	697 (24.5)	721 (25.3)	745 (26.2)	0.07

Data were presented as mean (SD), median (interquartile range), or proportions. Linear regression for continuous variables and Cochran-Armitage trend χ^2 test for categorical variables were applied to analyze the trends across T2D_GRS quartiles.

results. Compared with the lowest quartile of T2D_GRS, the second, third, and highest quartiles were associated with a 4, 8, and 19% increased risk of increased arterial stiffness, respectively, after adjustment for age, sex, BMI, and other covariates (*P* for trend = 0.02).

FPG and OGTT-2h PG levels were positively associated increased arterial stiffness (OR 1.07 [95% CI 1.02, 1.12] and 1.03 [1.004, 1.05] according to each 1 mmol/L increase of them, respectively), after adjustment for age, sex, BMI, current smoking, current drinking, physical activity, hypertension, serum TC, HDL cholesterol, LDL cholesterol, TG, and diabetes status. We further adjusted FPG and OGTT-2h PG levels or T2D status in models 3 and 4, respectively, to see if the relationship was mediated by hyperglycemia. We found that the association between T2D_GRS and increased arterial stiffness was not statistically significant after adjustments for FPG and OGTT-2h PG levels or T2D status (Table 2, models 3 and 4). The results indicated that the association of T2D_GRS with increased arterial stiffness might be mediated by hyperglycemia.

In the age-, sex-, and BMI-adjusted model, present T2D was associated with a 2.09-fold (95% CI 1.88, 2.32) increased risk of arterial stiffening risk. After further adjustment of other metabolic profiles, including glucose levels, the association was attenuated but remained significant (OR 1.28 [95% CI 1.08, 1.53]).

T2D and Increased Arterial Stiffness Risk: The MR analysis

Figure 1 shows the comparison of the observed association of T2D and risk of increased arterial stiffness with the IV causal estimator. For comparison, in the IV analysis, the causal OR of genetically determined T2D for increased arterial stiffness was 1.24 (95% CI 1.06, 1.47; *P* = 0.008). The causal estimate of the relationship between genetically determined T2D and increased arterial stiffness risk from the MR analysis was less than the observed association between T2D and increased arterial stiffness risk (1.24 vs. 1.78; $P < 0.0001$).

Association of Function-Specific GRS With Increase in baPWV

In the multivariable-adjusted linear regression model, we found that per SD (3.89 points) increment in T2D_GRS was associated with 9.20 cm/s higher baPWV (*P* = 0.003), and per SD (2.51 points) increase in IS_GRS was in

relation to 9.73 cm/s increase in baPWV (P = 0.002). For the IR_GRS, we did not find any positive linear association with baPWV (Fig. 2). In the MR analysis, we found that a decrease in IS, measured by HOMA-β, was causally associated with an increase in baPWV, in which genetically determined HOMA-β (per unit decrease in Log_{10}-HOMA-β) was in relation to a 122.3 cm/s higher baPWV (95% CI 41.9, 204.9; P = 0.0005) (Fig. 3*A*). With regard to IR, measured by HOMA-IR, we did not find any significant causal association with the higher baPWV (Fig. 3*B*).

Sensitivity Analysis

When we excluded those 16 SNPs related to other T2D-related metabolic traits, the T2D_GRS constructed by the SNPs only related to T2D (T2D_GRS/$_{18\ SNP}$) was not associated with those metabolic traits any more, except for the glucose metabolism–related variables (Supplementary Table 4). The T2D_GRS/$_{18\ SNP}$ was associated with increased arterial stiffness (in both continuous and categorical variable analysis) (Supplementary Table 5). The IV estimate for causal relationship between T2D and increased arterial stiffness was OR 1.24 (95% CI 1.004, 1.53; P = 0.02) (Supplementary Figs. 3 and 4).

We observed a significant causal association between genetically determined T2D and increased arterial stiffness with an OR of 1.21 (95% CI 1.03, 1.42; P = 0.01) using the unweighted T2D_GRS (Supplementary Fig. 3). Among the 34 SNPs of T2D, we found that *SPRY2* rs1359790 showed the strongest association with arterial stiffness (Supplementary Fig. 1). As shown in Supplementary Fig. 3, in a restricted analysis excluding the locus of *SPRY2*, the genetically determined T2D was still causally related to increased arterial stiffness (OR 1.18, 95% CI 1.01, 1.40; P = 0.04). When we included those individuals missing more than two variants of genotyping data as a missing indicator in the analysis, the results did not change appreciably.

DISCUSSION

In the cross-sectional investigation including almost 12,000 community-dwelling Chinese adults, we reported that the T2D_GRS, composed of 34 T2D common variants of East Asians, was significantly associated with increased arterial stiffness, which was assessed by elevated baPWV. In the MR analysis, T2D was causally associated with arterial stiffening. Our data for the first time provided novel evidence supporting a causal relationship between genetically determined T2D and arterial stiffening using the MR approach.

Cardiovascular disease is the main cause of death in T2D patients (32). Arterial stiffening may be one important pathway linking diabetes to the cardiovascular outcomes. Previous studies showed that the age-related increase in arterial stiffness is steeper in individuals with T2D than in the counterparts without diabetes. Ravikumar et al. (33) reported that patients with diabetes have increased arterial stiffness compared with age- and

Table 2—The associations of T2D_GRS and present T2D with increased arterial stiffness

		Model 1		Model 2		Model 3		Model 4	
	Patients/participants	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Continuous variable of T2D_GRS per SD (3.89 points)	2,853/11,385	1.06 (1.01, 1.11)	0.02	1.06 (1.01, 1.12)	0.01	1.03 (0.98, 1.08)	0.28	1.02 (0.98, 1.08)	0.33
Categorical variable of T2D_GRS									
Quartile 1	690/2,842	1.00		1.00		1.00		1.00	
Quartile 2	697/2,851	1.03 (0.90, 1.18)	0.02	1.04 (0.90, 1.20)	0.02	1.00 (0.86, 1.16)	0.21	0.99 (0.86, 1.15)	0.24
Quartile 3	721/2,846	1.07 (0.94, 1.23)		1.08 (0.93, 1.25)		1.02 (0.88, 1.19)		1.02 (0.88, 1.18)	
Quartile 4	745/2,846	1.16 (1.02, 1.33)		1.19 (1.03, 1.38)		1.10 (0.95, 1.27)		1.09 (0.94, 1.26)	
Present T2D									
	2,853/11,385	2.09 (1.88, 2.32)	< 0.0001	1.74 (1.54, 1.96)	< 0.0001	1.28 (1.08, 1.53)	0.004	—	—

Model 1, adjusted for age (years), sex, and BMI (kg/m^2); model 2, further adjusted for current smoking (yes or no), current drinking (yes or no), physical activity (mild, moderate, or vigorous), hypertension (yes or no), and serum TC (mmol/L), HDL cholesterol (mmol/L), LDL cholesterol (mmol/L), and TG (mmol/L); model 3, further adjusted for FPG and OGTT-2h PG based on model 2; and model 4, further adjusted for T2D status (yes or no) based on model 2.

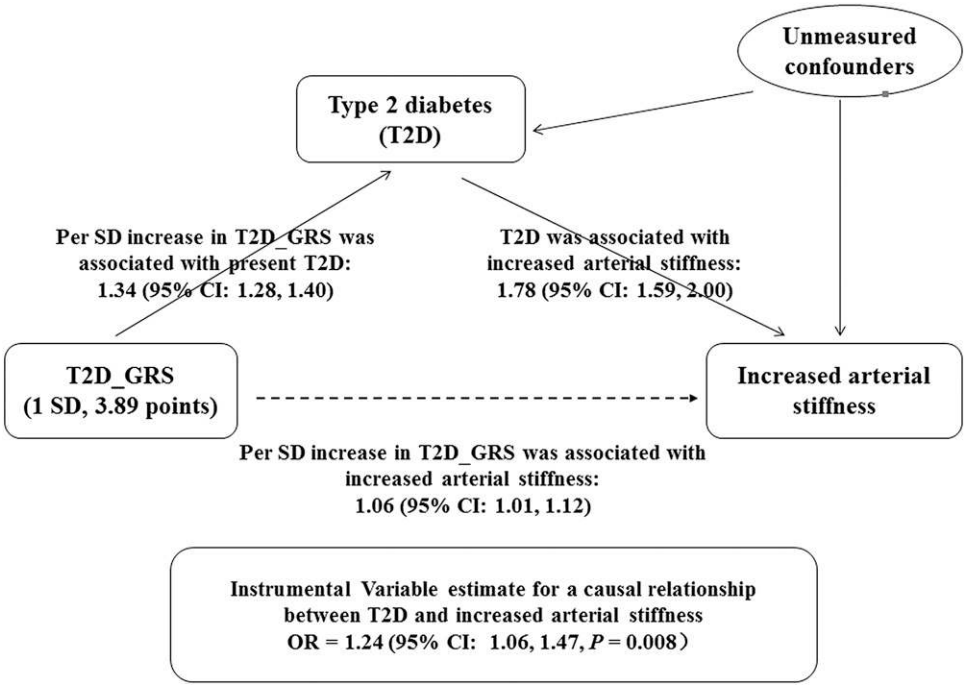

Figure 1—Observed vs. the IV estimated association of T2D and risk of increased arterial stiffness. In this MR framework, T2D_GRS–T2D association is assumed to be independent of the covariates. The IV estimator is ln (OR_{IV}) = Ln (1.06)/Ln (1.34), which equals a causal OR of T2D for increased arterial stiffness of 1.24 (95% CI 1.06, 1.47; P = 0.008). Data were adjusted for age, sex, BMI, current smoking, current drinking, physical activity, hypertension, and serum TC, HDL cholesterol, LDL cholesterol, and TG.

sex-matched subjects without diabetes. In addition, there was evidence that the presence of microvascular complications in T2D is associated with further increase in arterial stiffness (34,35). Despite this observational evidence, recent large-scale RCTs have shown conflicting and inconclusive results on the effect of intensive glucose-lowering therapies on the short-term prevention of cardiovascular diseases. Most studies have shown no benefit from intensive glucose-lowering therapy (9–12), but a recent meta-analysis of RCTs suggested a modest benefit

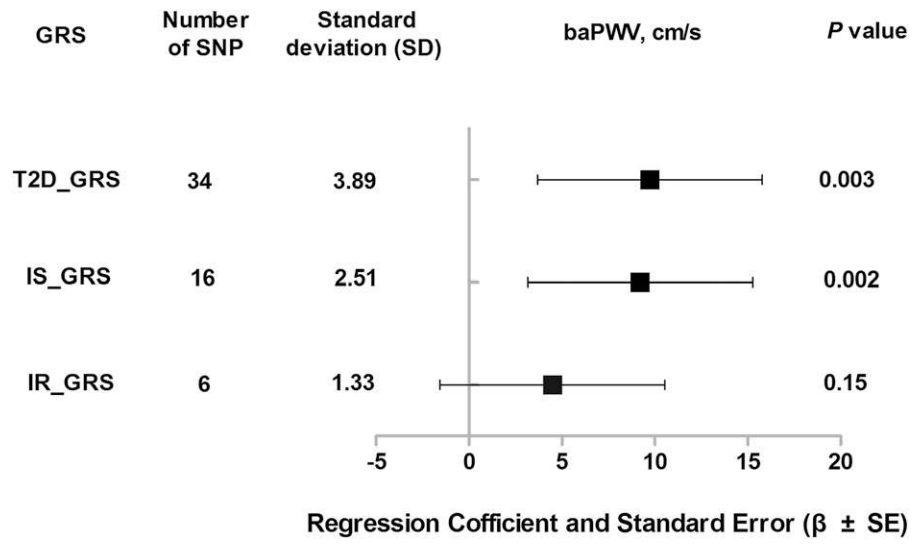

Figure 2—Associations of loci influencing IS and IR with baPWV. IS_GRS is composed of 16 SNPs that were significantly associated with Log_{10}-HOMA-β, and IR_GRS is composed of 6 SNPs that were significantly associated with Log_{10}-HOMA-IR. Data are presented as regression coefficient (β) and 95% CI, after adjustment for age, sex, BMI, current smoking, current drinking, physical activity, hypertension, and serum TC, HDL cholesterol, LDL cholesterol, and TG.

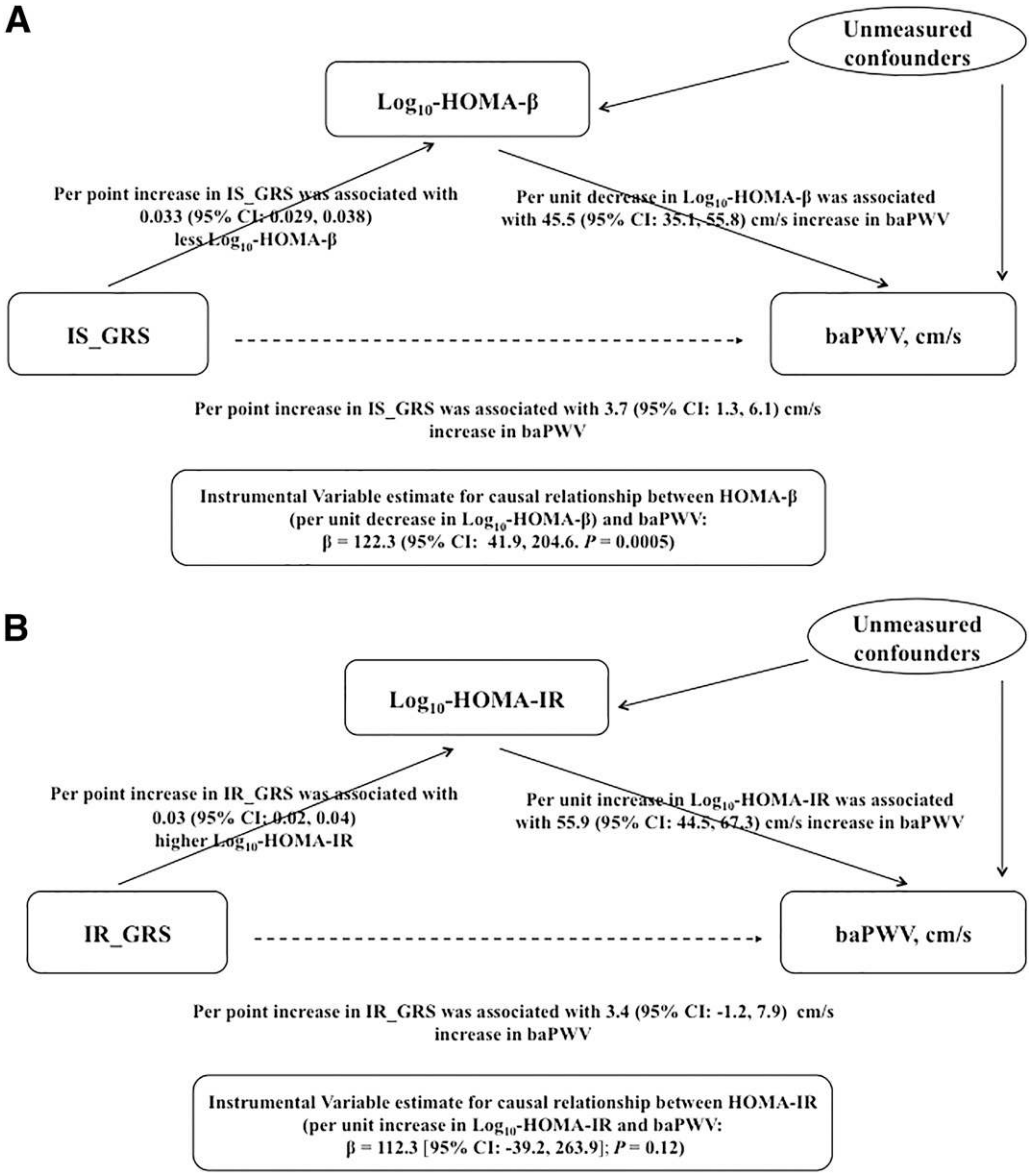

Figure 3—Observed vs. the IV estimated association of the IS_GRS (*A*) and the IR_GRS (*B*) with baPWV. Data are presented as regression coefficient (β) and 95% CI. All analysis were adjusted for age, sex, BMI, current smoking, current drinking, physical activity, hypertension, and serum TC, HDL cholesterol, LDL cholesterol, and TG.

of glucose lowering on cardiovascular outcomes (36). Obesity, hypertension, dyslipidemia, sedentary lifestyle, and stress are common shared risk factors that contribute to the association between T2D and cardiovascular diseases, which make it difficult to infer causality. Therefore, the use of the MR approach has been one helpful approach to figure out the bias matter and strengthening causal inferences between T2D and arterial stiffening.

Previously MR studies provided significant evidence to support causal links between biomarkers, traits, and diseases and cardiovascular diseases (37,38). Rasmussen-Torvik et al. (39) demonstrated a significant association of the FPG_GRS with intima-media thickness and suggested a possible causal association of elevated FPG with atherosclerosis. One previous MR study showed that non–FPG in individuals with diabetes is causally related to ischemic heart disease in a large group of Danish subjects (40). The latest MR studies have reported that diabetes_GRS was significantly associated with coronary artery disease and provided a causal relationship between them (41,42). Our study, for the first time using the MR approach, provided evidence that higher T2D_GRS was associated with increase in baPWV and demonstrated that genetically determined T2D may be causally related to the development of arterial stiffening.

MR study is a valid way to explore evidence for causality, given that certain assumptions are met (43). First, there has to be a strong association between the IV and risk

factor of interest. All SNPs used in this study have previously been shown to be strongly associated with T2D in a large meta-analysis of GWAS and could mostly be replicated in our present study. Second, the IV must be independent of the covariates. In our study, the T2D_GRS was found to be associated with the glucose metabolism traits, such as FPG and OGTT-2h PG levels and HOMA-β, but not with lipid measures, physical activity, smoking, or drinking, which are traditional important factors for cardiovascular disease. It was also found to be associated with sex distribution, BMI, and SBP. It might be due to the population stratification or different genes in linkage disequilibrium with the variants' use. However, to minimize the pleiotropic effect, we first examined the association of each SNP with other traditional factors related to arterial stiffness, such as BMI, blood pressure, and lipids, and created a T2D_GRS excluding those SNPs that associated with other metabolic traits. The IV estimated causal relationship remained significant. Given that FPG and OGTT-2h PG were both associated with the T2D_GRS and increased arterial stiffness, we then further adjusted for FPG and OGTT-2h PG or the T2D status in the association analyses. The association of T2D_GRS with increased arterial stiffness was dismissed after these adjustments. These results add further weight to the inference of these genes having a causal effect on baPWV via hyperglycemia and/or T2D as opposed to any unmeasured pleiotropic-related confounders. In addition, we performed sensitivity analysis using weighted and unweighted T2D_GRS and constructed a score without including the strongest loci in calculation. These three genetic scores yielded consistent results. Third, the genetic instrument is independent of the outcome, that is to say there is no direct effect of genotype on disease or any other mediated effect other than through the exposure of interest (no other routes in the path between T2D_GRS and arterial stiffening) (43). However, this assumption is largely untestable. It is possible that the association between genetically determined diabetes with increased risk of arterial stiffening is due to lifestyle choices, hyperglycemia, or other diabetes characteristics as a direct consequence of being diabetes. It is also possible that T2D genetic variants affect biological pathways, which, on the one hand, determine T2D and, on the other hand, influence the risk of arterial stiffening. Previous pathway analysis identified a number of overlapping pathways linking T2D-associated SNPs that could also have an effect on the development of atherosclerosis, including the NF-κB, STAT3, and IGF-1 pathways (44–46), all of which have experimentally documented roles in the risk of cardiovascular diseases.

Intriguingly, we found that the results were consistent when restricting the analysis to genetic variants affecting decreased IS but not IR. Given that the current T2D susceptibility loci identified from GWAS are mostly associated with β-cell dysfunction (47,48), our findings were expected. However, the large meta-analysis GWAS focused on IR was spare, only several validated loci associated with IR have been reported. We may have limited power to confirm whether there is a causal relationship between genetically determined IR and arterial stiffening.

The strengths of our study were its well-defined community setting, a relative large sample size, the MR study design, and a created T2D_GRS representing the combined effect of the established common genetic variations of T2D as the IV. In our study, we chose baPWV as the marker of arterial stiffness. The validity and reproducibility of baPWV measurements are considerably high. This simple, noninvasive method is now an acceptable marker reflecting vascular damages and is suitable for screening vascular damages in a large population (19). However, there are several limitations we should acknowledge. First, we built up our T2D_GRS only based on common variants, which was considered to represent limited diabetes heritability. We were unable to assess the potential contribution of rare variants. Second, it is still difficult to use T2D_GRS to completely discriminate the effects of T2D from other cardiovascular risk factors on risk of increased arterial stiffness due to possible pleiotropic effects of SNPs in the T2D_GRS. However, it is not expected that all the SNPs used to construct the score would have similar pleiotropic effects as each SNP acts on T2D independently and via different pathways. Third, although this study provided insights into the likely causal effect of lifetime exposure of hyperglycemia, we could not comment on the impact of acute changes. Finally, we created the T2D_GRS by using the common variants that were robustly associated with T2D in East Asians. We need to be cautious in generalizing the findings to other ethnicity groups.

In conclusion, we found a higher T2D_GRS was associated with higher risk of increased arterial stiffness. This analysis has enabled us to provide evidence for the biologically plausible causal relationship between genetically determined T2D and arterial stiffening. Our study suggested that long-term treatment with T2D may be beneficial for preventing the development of arterial stiffening. Additional prospectively designed studies are needed to validate our findings in other cohorts.

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