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Obesity and peripheral arterial disease: A Mendelian Randomization analysis

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ABSTRACT

Background and aims: Observational studies showed that obesity is a major risk factor for peripheral arterial disease (PAD). However, conventional epidemiology studies are vulnerable to residual bias from confounding factors. We aimed to explore the causality of obesity in development of PAD using Mendelian Randomization (MR) approach.

Methods: A MR analysis was performed in 11,477 community-dwelling adults aged 40 years and above recruited from two nearby communities during 2011–2013 in Shanghai, China. We genotyped 14 body mass index (BMI) associated common variants identified and validated in East Asians. PAD was defined as ankle-to-brachial index (ABI) <0.90 or >1.40. Weighted BMI genetic risk score (GRS) was used as the Instrumental Variable (IV).

Results: After adjusted for confounding factors, we found that each standard deviation (SD, 2.76 points) increase in BMI-GRS was associated with 0.43 (95% confidence interval [CI]: 0.36–0.49) kg/m^2 increase in BMI ($P < 0.0001$) and an odds ratio (OR) for PAD of 1.17 (95% CI: 1.07–1.27; $P = 0.0004$). Compared with the lowest quartile of BMI-GRS, the second, third and highest quartile associated with 9%, 19% and 45% increment of PAD risk, respectively (P for trend = 0.002). In the MR analysis, we demonstrated a causal relationship between obesity and PAD (OR = 1.44 per BMI-unit, 95% CI: 1.18–1.75; $P = 0.0003$).

Conclusions: This study suggested that obesity may be causally associated with PAD after controlling for the potential intermediate factors like hypertension, dyslipidemia and hyperglycemia.

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1. Introduction

Peripheral arterial disease (PAD), which is characterized by narrowing and blockade of peripheral arteries [1], affects almost 10% people worldwide and nearly 15–20% in people over 70 years old [2]. The most severe manifestation of PAD, critical limb ischemia, can lead to limb loss and even death if not treated promptly [2]. Previous analysis suggested that obesity is an independent risk factor for cardiovascular events in patients with PAD [3]. Several observational prospective investigations showed a significant relationship between obesity, indicated as body mass index (BMI) and the risk of PAD [4–7]. However, observational association of BMI and PAD is subject to a variety of bias such as confounding [8] and reverse causation [9], which making it difficult to infer causality from the observed associations.

In recent years, the "Mendelian Randomization" (MR) analysis

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using genetic variants as the instrumental variable (IV) has been widely used for assessing causality in the cardiovascular risk epidemiological studies [10–13]. Genetic alleles are allocated randomly during gamete formation; and the common variants are inherited independent of potential confounding factors [12,13]. Therefore, the IV using an independent genetic factor is regarded as independent of confounders in affecting the intermediate phenotype (BMI in the present analysis for instance)-outcome relationship [14,15]. Thus, the discovery of genetic variants reproducibly associated with BMI provides the opportunity to explore a causal association between BMI and risk of PAD. However, in some cases, there may be no variants which are solely associated with the risk factor of interest, and a MR analysis cannot be performed without considering the pleiotropy [16,17]. This limitation can be averted by adopting the method proposed by Do R [18] to adjust MR analysis for genetic effects on these other risk factors.

In order to reduce statistical errors with multiple testing, to create a genetic variable that accounted for a substantive amount of variation, a composite genetic risk score (GRS) was more advantaged [19]. BMI-GRS, which may represent a combined genetic effect of BMI, possibly will present the obesity susceptibility. In the present study, we aimed to test the association of BMI-GRS and risk of PAD in a large sample of Chinese population, and to explore the causal association between BMI and PAD using the MR approach.

2. Materials and methods

2.1. Population

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 and older. Details of the study rationale and profile have been published elsewhere [20,21]. The participants in the present study were recruited from two nearby communities at Baoshan district in the city of Shanghai during 2011 and 2013. Briefly, a standard questionnaire was used to collect information about lifestyle factors, disease and medical history. Anthropometric measurements, 75-g oral glucose tolerances test (OGTT) and blood and urine sampling were performed.

There were 11,935 participants (average age 63.5 years and 35.6% men) were recruited in the study, in which genotype information was available in 11,837 participants (99.2%). Individuals with missing information on ankle-brachial index (ABI) ($n = 143$) or BMI ($n = 17$) were excluded. The Institutional Review Board of Rui-Jin Hospital, Shanghai Jiao Tong University School of Medicine, approved the study protocol. Written informed consent was obtained from each participant.

2.2. Anthropometric information and laboratory measurements

A questionnaire was used to collect the social demographic information, the history of chronic diseases, use of medications and lifestyle factors, such as tobacco smoking and alcoholic drinking habits. The current smoking or drinking status was defined as “yes” if the subject smoked cigarettes or consumed alcohol regularly in the past 6 months [20,21]. The trained investigators measured body height and body weight. BMI was calculated as body weight in kilograms divided by height squared in meters (kg/m^2). Overweight was defined as 25 ≤ BMI <30 kg/m^2 and obesity was defined as BMI ≥30 kg/m^2 according to the World Health Organization (WHO) criteria [22]. Systolic and diastolic blood pressure (SBP and DBP) were measured by using an automated electronic device (OMRON Model HEM-752 FUZZY, Omron Company, Dalian, China) in triplicate on the same day after at least ten-min's rest, 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 be evaluated the biomarkers at the same laboratory. Fasting and 2-h plasma glucose (FPG and 2h-PG) were measured by using hexokinase method on a clinical chemistry diagnostic system (C16000, Abbott Laboratories, Otawara-shi, Japan). Serum concentrations of triglyceride (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-cholesterol) and low density lipoprotein cholesterol (LDL-cholesterol) were measured by using an autoanalyser (ADVIA-1650 Chemistry System, Bayer Corporation, Germany). Serum fasting insulin was measured by using the immunoassay diagnostic system (I2000, Abbott Laboratories, Dallas, USA). Computer homeostasis model assessment of insulin resistance (HOMA2-IR) [23], which consists of a number of nonlinear empirical equations accounts for variants in hepatic and peripheral glucose resistance, was used to determine the level of insulin resistance.

2.3. Diagnosis of PAD

A fully automatic arteriosclerosis diagnosis device (Colin VP-1000, ModelBP203RPE II, form PWV/ABI) was used to measure the ABI with the participants in the supine position after resting for 10–15 min. Participants who had an ABI <0.9 or >1.4 at either side were diagnosed as having PAD [24].

2.4. Selection of genetic loci, genotyping, and GRS construction

We selected 14 common single nucleotide polymorphisms (SNPs) from 14 established loci that associated with BMI in recently published genome wide association studies (GWASs) in East Asians, including: *FTO* rs17817449, *MC4R* rs6567160, *GNPDA2* rs10938397, *BDNF* rs6265, *SEC16B* rs574367, *TFAP2B* rs4715210, *MAP2K5* rs4776970, *GIPR-QPCTL* rs11671664, *ADCY3-DNAJC27* rs6545814, *CDKAL1* rs9356744, *PCSK1* rs261967, *GP2* rs12597579, *PAX6* rs652722 and *SMC5-KLF9* rs11142387 [25,26]. They all reached a genome-wide significance level ($P < 5 \times 10^{-8}$) and no linkage disequilibrium relationship existed among the above loci. For the GRS construction, we created two kinds of scores, one was un-weighted GRS and the other was weighted GRS. For un-weighted GRS, we assumed the additive genetic model [19] for each SNP, applying a linear weighing of 0, 1 and 2 to genotypes containing 0, 1 or 2 risk alleles, respectively. We excluded the participants who were missing more than two SNPs ($n = 298$). Thus, a total of 11,477 subjects were included in the final analysis. With those who were missing one or two SNPs, we assigned them the average genetic score. Using these 14 SNPs, we constructed an un-weighted GRS ranging from 2.00 to 20.46 on the basis of the number of risk alleles; and a weighted genotype score ranging from 1.30 to 19.96 based on weighting each allele with the effect size (β) of association with BMI summarized in the literature [25,26] and Supplemental Table 1. All the results in the present study were based on the weighted genetic score, and the un-weighted genetic score was used in the sensitivity analysis.

Blood white cells were collected for DNA extractions by 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 manufacturer's standard protocol. Specific assays were designed using the MassARRAY Assay Design software package (v3.1). Mass determination was carried out with the MALDI-TOF mass spectrometer and Mass ARRAY Type 4.0 software was used for data acquisition (SEQUENOM, CapitalBio Corporation, Beijing, China). Genotyping was performed in each

subject. The minimum call rate was 98.7%. The concordance rate is more than 99% based on 100 duplicates genotyping.

Almost all the SNPs were in Hardy–Weinberg equilibrium, except for *TFAP2B* rs4715210 and *GP2* rs12597579 ($P = 0.02$ and 0.04, respectively).

2.5. Statistical analysis

SAS version 9.3 (SAS Institute, Cary, NC) was used for database management and statistical analysis. Continuous variables with normal distribution were given as means ± standard deviation (SD) and with skewed distribution were given as medians (inter-quartile ranges). Serum TG and HOMA2-IR were normalized by logarithmic transformation because of skewed distributions if necessary statistically. Categorical variables were shown in proportions. All the participants were divided into four groups based on BMI-GRS. Continuous variables in each quartile were described and linear regression analysis was used to test for the trend across the BMI-GRS quartiles. Cochran–Armitage trend chi-square test was used for categorical variables. Multiple linear regression analysis was used to evaluate the linear association of BMI-GRS with BMI and other metabolic profiles. Multivariate logistic regression models were used to assess the risk of PAD related to BMI-GRS and BMI, which was evaluated as continuous variable (each SD), or categorical variables (the higher quartiles versus the lowest quartile, the lowest quartile as the reference group).

In the MR analysis, we used the IV estimator to measure the strength of the causal relationship between BMI and risk of PAD. The BMI-GRS was chosen as the IV. The IV estimate of causal odds ratio (OR) was derived by using the Wald-type estimator [27] and then exponentiating to express as an OR. The computational formula was $OR_{IV} = \exp(\text{Ln}(OR_{GRS\text{-}PAD})/\beta_{GRS\text{-}BMI})$. For PAD risk, we tested the null hypothesis of no difference between the IV estimator and the conventional regression-based estimator for the effect of BMI via a classical z-test.

Statistical significance was set to a two-sided P value of less than 0.05.

3. Results

3.1. Characteristics of study participants

Among the 11,477 participants, 4070 (35.5%) were men. The average age was 63.2 (SD 9.6) years and the average BMI was 25.26 (SD 3.60) kg/m^2. The number (proportion) of PAD patients was 701 (6.1%), including 316 men and 385 women suffered from PAD.

The characteristics of individual SNPs in the BMI-GRS and the association of each BMI-SNP with BMI were shown in Supplemental Table 1. The distribution of BMI-GRS was shown in Fig. 1 and the mean value was 10.15 (range from 1.30 to 19.96) with a SD of 2.76. Table 1 demonstrated the demographic and metabolic features of the participants according to BMI-GRS quartiles. The BMI-GRS (in quartiles) was significantly associated with BMI (P for trend < 0.0001). Male sex, status of current smoking, SBP, DBP, FPG, TG, TC, LDL-cholesterol, HDL-cholesterol and HOMA2-IR increased significantly with incrementing BMI-GRS quartiles (all P for trend < 0.05). No difference of 2h-PG and status of current drinking were found among quartiles.

3.2. Association of BMI-GRS and BMI with risk of PAD

As shown in Table 2, each SD (2.76 points) increase in BMI-GRS was associated with 25% increased risk of PAD after adjusted for age and sex (OR = 1.25, 95% CI: 1.15–1.35, $P < 0.0001$). When stratified by sex, each SD (2.76 points) increment in BMI-GRS was associated with 33% increased risk in PAD (OR = 1.33, 95% CI: 1.18–1.49, $P < 0.0001$) in men, and 20% in women (OR = 1.20, 95% CI: 1.08–1.33, $P = 0.0006$).

Further adjustment for smoking and drinking status, blood pressure, plasma glucose, and serum lipids did not substantially change the results (OR = 1.17, 95% CI: 1.07–1.27, $P = 0.0004$). Moreover, further adjustment for BMI attenuated but retained statistically significance (OR = 1.14, 95% CI: 1.04–1.24, $P = 0.003$). The categorical analysis showed similar results. Compared with the lowest quartile of BMI-GRS, the second, third and highest quartiles were associated with a 9%, 19% and 45% increased risk of PAD, respectively, after adjusted for the confounders (P for trend = 0.002).

In multivariable adjusted model, each SD (3.60 kg/m^2) increase in BMI was associated with a 23% (95%CI: 1.13–1.33, $P < 0.0001$) incremental PAD risk. Similarly, the categorical analysis showed that compared with the lowest quartile of BMI, the highest quartile was associated with a 31% (95% CI: 1.03–1.67) increased PAD risk (P for trend = 0.008).

3.3. BMI and PAD risk: the MR analysis

Fig. 2 showed the comparison of the observed association of BMI and risk of PAD with the IV causal estimator. When adjusted for age, sex, current smoking, current drinking, blood pressure, plasma glucose and serum lipid profiles, each SD (2.76 points) increase in BMI-GRS was associated with 0.43 (95% CI: 0.36–0.49, $P < 0.0001$) kg/m^2 increment in BMI and an odds ratio (OR) for PAD of 1.17 (95% CI: 1.07–1.27, $P = 0.0004$). The observational analysis showed that each unit increase in BMI (kg/m^2) associated with 1.06-folds risk for PAD (95% CI: 1.03–1.08, $P < 0.0001$) in multivariable adjusted model. For comparison, in the IV analysis, the causal OR of one kg/m^2 increase in BMI for PAD was 1.44 (95% CI: 1.18–1.75, $P = 0.0003$). The causal estimate of the relationship between BMI and PAD risk from the MR analysis was greater than the observed association between BMI and PAD risk (1.44 versus 1.06, $P < 0.0001$).

3.4. Association of BMI-GRS with BMI and other metabolic features

Multiple linear regression analysis demonstrated that the BMI-GRS was associated with BMI, SBP, DBP, FPG, log-TG, TC, LDL-cholesterol, HDL-cholesterol and log-HOMA2-IR in the age- and sex-adjusted model (all P values < 0.001, Supplemental Table 2). After further adjusted for BMI, BMI-GRS was still significantly associated with DBP, FPG, TC, LDL-cholesterol and HDL-cholesterol (all P values < 0.05).

3.5. Sensitivity analysis

In the sensitivity analysis (Supplemental Fig. 1), we observed a significant causal association between obesity and risk of PAD with an OR of 1.42 (95% CI: 1.17–1.73, $P = 0.0005$) using the un-weighted GRS of BMI. Considering the pleiotropy, we constructed a GRS excluding the most obvious loci maybe involved in other traits regulation, *FTO* rs17817449 and *MC4R* rs6567160 [28], and found that genetically determined obesity was still causally in relation to risk of PAD (OR = 1.62, 95% CI: 1.30–2.00, $P < 0.0001$).

4. Discussion

In the large population study of 11,477 Chinese adults, we reported that the BMI-GRS of East Asians significantly associated with the risk of PAD. The MR analysis suggested that elevated BMI was causally associated with prevalence of PAD. Our data for the first time provided novel evidence for a causal relationship between

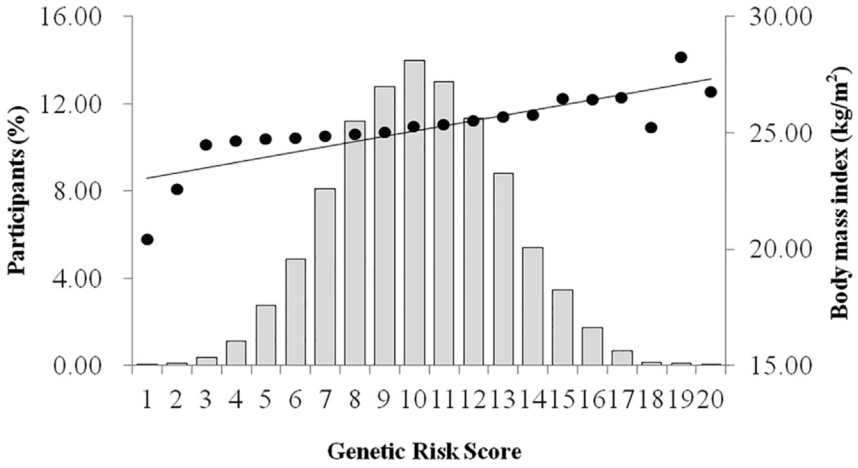

Fig. 1. Distributions of the weighted body mass index genetic risk score (BMI-GRS) and mean BMI level by the weighted BMI-GRS.

Table 1
Characteristics of study population by weighted BMI-GRS quartiles.

Characteristics	BMI-GRS				*P* for trend
	Quartile 1 (n = 2861)	Quartile 2 (n = 2873)	Quartile 3 (n = 2872)	Quartile 4 (n = 2871)	
BMI-GRS	6.64 ± 1.21	9.18 ± 0.55	11.06 ± 0.56	13.69 ± 1.32	/
Age (years)	64.2 ± 10.2	63.3 ± 9.7	63.1 ± 21.0	62.9 ± 21.7	<0.0001
Male sex, *n* (%)	966 (33.8)	995 (34.6)	1079 (37.6)	1033 (36.0)	0.02
Body mass index (kg/m^2)	24.75 ± 3.46	25.09 ± 3.59	25.34 ± 3.56	25.88 ± 3.71	<0.0001
Systolic blood pressure (mmHg)	136 ± 21	136 ± 20	137 ± 21	138 ± 20	<0.0001
Diastolic blood pressure (mmHg)	76 ± 10	77 ± 10	77 ± 10	78 ± 10	<0.0001
Fasting plasma glucose (mmol/L)	5.84 ± 1.44	5.96 ± 1.68	6.07 ± 1.88	6.14 ± 1.87	<0.0001
2h plasma glucose (mmol/L)	8.80 ± 3.84	8.83 ± 4.09	8.97 ± 4.25	8.96 ± 4.25	0.12
HOMA2-IR (%)	0.81 (0.58–1.14)	0.82 (0.59–1.15)	0.86 (0.61–1.22)	0.94 (0.64–1.23)	<0.0001
Triglyceride (mmol/L)	1.26 (0.91–1.80)	1.26 (0.91–1.79)	1.30 (0.94–1.84)	1.30 (0.95–1.86)	<0.0001
Total cholesterol (mmol/L)	4.61 ± 1.18	4.84 ± 1.19	5.08 ± 1.17	5.24 ± 1.10	<0.0001
LDL-cholesterol (mmol/L)	2.67 ± 0.86	2.82 ± 0.88	2.97 ± 0.89	3.07 ± 0.86	<0.0001
HDL-cholesterol (mmol/L)	1.15 ± 0.33	1.20 ± 0.34	1.23 ± 0.33	1.26 ± 0.32	<0.0001
Current smoking, *n* (%)	344 (12.4)	375 (13.7)	454 (16.9)	450 (17.0)	<0.0001
Current drinking, *n* (%)	252 (9.1)	243 (8.9)	273 (10.2)	258 (9.8)	0.17
Overweight, *n* (%)	1051 (36.7)	1125 (39.2)	1230 (42.8)	1305 (45.5)	<0.0001
Obesity, *n* (%)	212 (7.4)	242 (8.4)	252 (8.8)	346 (12.1)	<0.0001
PAD, *n* (%)	140 (4.9)	159 (5.5)	180 (6.3)	222 (7.7)	0.0001

BMI-GRS: body mass index genetic risk score; HOMA2-IR: computer homeostasis model assessment of insulin resistance; PAD: peripheral arterial disease.
Data are presented as means ± standard deviation (SD), medians (inter-quartile ranges), or number (proportions). Overweight was defined as 25 ≤ BMI <30 kg/m^2 and obesity was defined as BMI ≥30 kg/m^2 according to the World Health Organization (WHO) criteria. PAD was defined as ankle-brachial index (ABI) < 0.9 or > 1.4 either side.

obesity and PAD.

PAD, a manifestation of generalized atherosclerosis, is known to affect quality of life and associated with higher risk of cardiovascular morbidity and mortality [29]. Obesity is thought to be an independent risk factor for PAD. In epidemiology studies, high BMI has been consistently related to an increased risk of PAD. However, the causal role of obesity in development of PAD has yet to be established.

MR analysis is a recent improvement in genetic epidemiology [14]. The independent distribution of alleles/genotypes means that the association of a health outcome with a genetic variation would not be affected by confounders that often distort the interpretation of findings [30]. Moreover, the random assignment of alleles/genotype transferred from parent to offspring at the time of gamete formation. Because of the random assignment to genotype takes place at conception, the association between a genetic variant and a disease is largely free of reverse causation. Besides, MR study is linked to a natural randomized controlled trial (RCT) according to methodology. The MR approach may have particular superiority in assessing the effects of long-term or lifetime exposures, such as lifestyle or obesity, whereas conventional RCTs only can examine short-term effects [30]. Above all, using MR approach has been a preferable choice in causality inference investigation. Previously MR studies provided significant evidence to support causal links between increased BMI and cardiometabolic disorders and traits, such as type 2 diabetes [31], hypertension [32], ischemic heart diseases [33], heart failure [11,31] and cardiometabolic traits such as blood pressure, plasma glucose, serum lipids, liver enzymes and inflammation markers [11]. Most of the previous MR analysis used a single locus, such as *FTO* or *MC4R* as the IV [11,31–33], and was conducted in Caucasians.

However, a single genetic variant typically explains a small proportion of the variability of the risk factors; hence MR analysis requires large sample size unless the instrument is strongly associated with phenotype of interest. Multi-gene genetic risk score is important for the modeling of polygenic diseases or traits. When several variants are combined into a genetic risk score, the score may explain a considerable proportion of variation in the risk factor, even if none of the variants individually does. Using score of multiple genetic variants can improve the power of statistical analysis

Table 2
The association of weighted BMI-GRS and BMI with risk of PAD.

	Model 1		Model 2		Model 3	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Continuous, per 1-SD (2.76 points) BMI-GRS						
	1.25 (1.15–1.35)	<0.0001	1.17 (1.07–1.27)	0.0004	1.14 (1.04–1.24)	0.003
Categorical, quartiles of BMI-GRS						
Q1	1.00		1.00		1.00	
Q2	1.18 (0.93–1.49)		1.09 (0.85–1.39)		1.07 (0.83–1.37)	
Q3	1.35 (1.07–1.70)		1.19 (0.93–1.53)		1.16 (0.91–1.48)	
Q4	1.73 (1.39–2.15)		1.45 (1.14–1.84)		1.36 (1.07–1.73)	
P for trend	<0.0001		0.002		0.009	
Continuous, per 1-SD (3.60 kg/m^2) BMI						
	1.33 (1.24–1.43)	<0.0001	1.23 (1.13–1.33)	<0.0001	/	/
Categorical, quartiles of BMI						
Q1	1.00		1.00		/	
Q2	0.98 (0.77–1.24)		0.87 (0.68–1.13)		/	
Q3	1.17 (0.93–1.48)		1.01 (0.79–1.30)		/	
Q4	1.75 (1.41–2.17)		1.31 (1.03–1.67)		/	
P for trend	<0.0001		0.008		/	

BMI: body mass index; GRS: genetic risk score; PAD: peripheral arterial disease; Q1: quartile 1; Q2: quartile 2; Q3: quartile 3; Q4: quartile 4.
Data are presented as odds ratio (OR), 95% confidence interval (CI).
P values were calculated from multivariable logistic regression analysis.
Model 1, adjusted for age and sex.
Model 2, additionally adjusted for current smoking and drinking status, systolic blood pressure, diastolic blood pressure, fasting plasma glucose, 2h-plasma glucose, total cholesterol, triglyceride, LDL-cholesterol and HDL-cholesterol based on model 1.
Model 3, was additionally adjusted for BMI based on model 2.

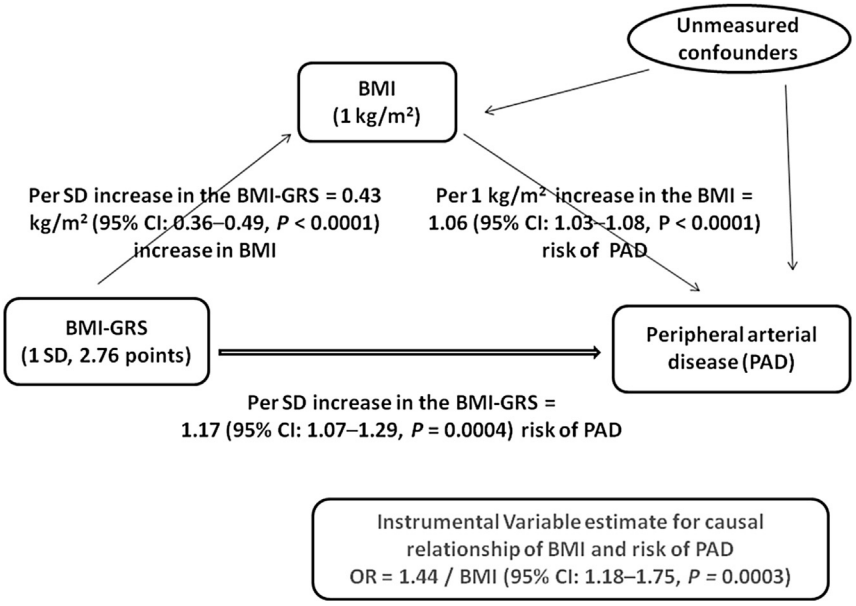

Fig. 2. Observed versus the IV estimated association of body mass index (BMI) and risk of peripheral artery disease (PAD). When adjusted for age, sex, current smoke, current drink, physical activity, blood pressure, plasma glucose and serum lipid profiles, the relationship of per 1 kg/m^2 increment in BMI with 1.06-fold risk of PAD, where the instrumental variable estimator is ln (OR_{IV}) = ln (1.17)/0.43, which equals a causal OR of BMI (1 kg/m^2) for PAD is 1.44 (95% CI: 1.18–1.75, P = 0.0003).

and facilitate tests of the IV assumption that are not possible in single instrument analysis [19,34]. Nevertheless, there is concern that if the multi-gene genetic risk score could be used as the IV, for each of the SNPs in the score may involve in different biological mechanisms. With respect to the present study, an increasing number of genetic variants have been found to be robustly associated with BMI in genome-wide association studies. The studies on mechanisms of most of these susceptibility genes affecting obesity in vivo or in vitro were still limited. The existing evidence was mainly focused on the regulation of energy homeostasis (*FTO, PCSK1*) [35,36], or dietary fat intake and fat accumulation (*BDNF, CDKAL1*) [37,38], or modification of satiety, depressed mood, or binge eating behaviors (*MC4R*) [39]. Given that each SNP acting on BMI independently and via different pathways, the score of multi-gene genetic variants may represent more comprehensive genetic predisposition of obesity. In this study, we provided novel evidence of causal association of obesity on risk of PAD in Chinese for the first time, by using a genetic score that combined 14 loci of BMI.

MR study is a valid way to explore evidence for causality, given that certain assumptions are met [40]. Firstly, the association of the IV used in the MR studies with the exposures of interest should be reliably demonstrated. All SNPs used in this study have previously

been shown to be strongly associated with BMI in large meta-analysis of GWASs in East Asians and could mostly be replicated in our present study. Secondly, the IV must be independent of covariates. However, a genetic variant may cause multiple biological alterations, i.e., pleiotropy [30]. We performed multivariable adjusted MR analysis proposed by Do R [18] to minimize this limitation. Thirdly, there is no direct effect of genotype on disease or any other mediated effect other than through the exposure of interest. However, this assumption is largely un-testable. It is possible that the association between genetically determined BMI with increased risk of PAD is due to lifestyle choices, hyperglycemia, hypertension or dyslipidemia as a direct consequence of being obesity. It is also possible that the associations of the genetic variants with BMI and with PAD are through completely different mechanisms. Although our analysis provided insight into the likely causal effect of genetic determined elevations in BMI on risk of PAD, we are not well to comment on the actual mechanisms. Further pathway analyses in large meta-analysis of GWASs or basic studies are warranted for the verifying of the assumption.

The strength of our study were its well-defined community setting, a relative large sample size, the MR study design and a created weighted BMI-GRS representing the combined effect of the established common genetic variations as the IV. However, there were several limitations we should acknowledge. Firstly, we build up our BMI-GRS only based on common variants, which was considered to represent part of the obesity heritability. We were unable to assess the potential contribution of rare variants. Secondly, it is difficult to use the BMI-GRS to completely discriminate the effects of obesity from other cardiovascular risk factors on risk of PAD because of possible pleiotropic effects of SNPs in the BMI-GRS [15]. Thirdly, though this study provided a likely causal effect of lifetime exposure of elevation in BMI on PAD, it cannot account for the effect of BMI changes in a short period. Fourthly, we diagnosed PAD according to ABI more than 1.40 or less than 0.90 either side [24]. While there was no direct PAD data, ultrasound based or clinically based, which was not easily applicable in large epidemiological studies. Lastly, we created the BMI-GRS using the common variants robustly associated with BMI in East Asians; it needs to be cautious to generalize the finding to other ethnicities or ethnic groups.

In conclusion, we found that a higher BMI-GRS was associated with higher risk of PAD; and by using the MR approach, this analysis has enabled us to provide evidence for the biologically plausible causal relationship, the obesity and PAD. However, considering the pleiotropic effects of the genes, result from the genetic variants based on GRS should be interpreted with caution. Additional studies are needed to elucidate the mechanisms behind these associations.

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Conflict of interest statement

None declared.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.atherosclerosis.2015.12.034.

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