Obesity and peripheral arterial disease: A Mendelian Randomization analysis

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ARTICLE INFO

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.

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 pheno-type (BMI in the present analysis for instance)-outcome relation-ship [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 advan-taged [19]. BMI-GRS, which may represent a combined genetic ef-fect 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 lONgitu-dinal (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 fac-tors, 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 infor-mation 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 ob-tained from each participant.

2.2. Anthropometric information and laboratory measurements

A questionnaire was used to collect the social demographic in-formation, 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 $25 \leq BMI <30$ 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 choles-terol (TC), high density lipoprotein cholesterol (HDL-cholesterol) and low density lipoprotein cholesterol (LDL-cholesterol) were measured by using an autoanalyser (ADVIA-1650 Chemistry Sys-tem, 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 al-leles; 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