Investigating the causal factors of peripheral arterial disease: a Mendelian Randomization study

Momoko Horikoshi
RIKEN
Centr for Integrative Medical Sciences
Laboratory for Genomics of Diabetes and Metabolism

1. Background

The most common cause of Peripheral arterial disease (PAD) is atherosclerotic vascular disease and is affecting more than 200 million people worldwide. Prevalence of PAD in East Asian adult is relatively low (1~3%) and higher prevalence is seen in male, elderly and African descent. Other known risk factors are smoking, diabetes, hyperlipidemia and hypertension. However, predisposition to PAD may be influenced by genetic variants acting independently of these risk factors. In contrast to coronary heart disease, relatively few genetic variants that influence susceptibility to PAD have been discovered. This may be, in part, because of greater clinical and genetic heterogeneity in PAD.

2. Aims

Our aim was to:

i. Assess the genetic background of peripheral arterial disease (PAD) in Japanese.
ii. Investigate the causal relationship between exposure (risk factors) and outcome (PAD) by using Mendelian Randomisation method.

3. Methods and Results

i. Genome-wide association analysis of PAD in Japanese

- Study participants

Samples and data were obtained from the Biobank Japan (BBJ) Project. Participants for the BBJ project were recruited at 66 collaborating hospitals throughout Japan. During the first period of the recruitment, 199,998 patients with at least one of the 47 common diseases were recruited and 2,824 PAD cases were included in the recruitment. PAD was diagnosed by the doctors at each recruiting hospital. Patients diagnosed with one of the following diseases were excluded from controls: diabetes, myocardial infarction, stroke, heart failure, stable and unstable angina and dyslipidemia.

- Genotyping and data cleaning

Samples were genotyped on Illumina HumanOmniExpressExome BeadChips. Samples with call rate <0.98, related samples (determined by PI_HAT >0.1 which is an index of relatedness between two individuals based on identity-by-descent (IBD)) and population other than Japanese (determined through principal component analysis (PCA)) were excluded from the study. As for the variant quality control, variants were excluded on the following condition: (1) heterozygosity count <5; (2) Hardy-Weinberg equilibrium P <$1.0\text{x}10^{-6}$ in each chip and (3) concordance rate <0.99 with in-house whole-genome sequence data using overlapping samples.

Principal components were recalculated using the cleaned samples and variants. Cleaned genotype data with minor allele frequency (MAF) >0.01 were prephased using EAGLE v2.3 and were imputed up to the 1000 Genomes Project Phase 3 reference panel (May 2013 release, all samples) with minimac3.

▪ Genome-wide association analysis (GWAS) for PAD

Association with PAD was tested in 2,365 PAD cases and 80,914 controls at 8,630,712 autosomal variants which had good imputation quality (defined by mimac r^2>=0.3). Association analysis was conducted using mach2dat (1.0.24) under additive model with adjustment for age, sex, 10 principal components and disease status. We detected five association signals reaching genome-wide significance (rs9582281, rs12713857, rs116234385, rs57301765 and rs1610914, P <5.0x10^{-8}) of which one at chromosome 7 was previously reported (Figure 1).

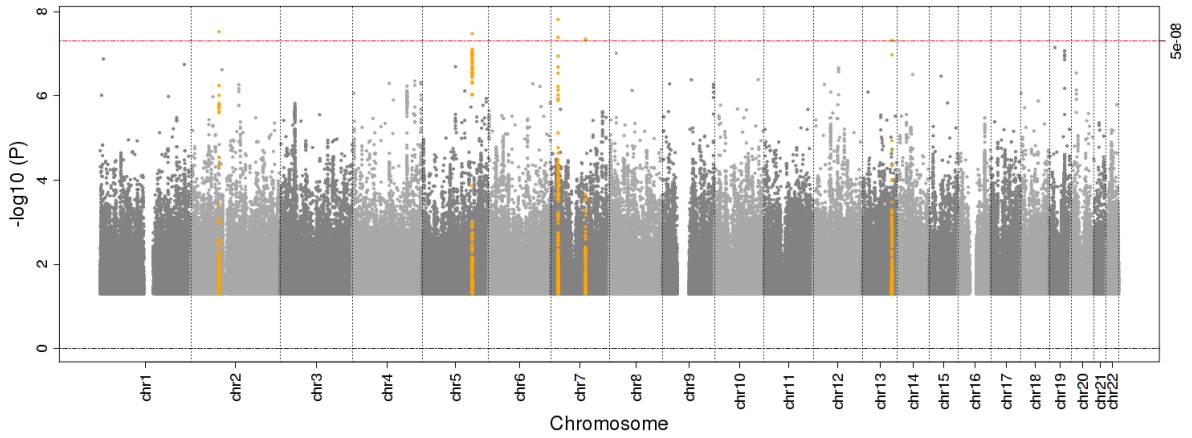


Figure 1. Genome-wide association results for PAD combining 2,365 cases and 80,914 controls in Japanese. $-\log_{10}(P)$ for each of up to 8,630,712 autosomal variants (y axis) was plotted against genomic position (NCBI Build 37; x axis). Association signals that reached genome-wide significance (P <5.0x10^{-8}) are shown in yellow.

ii. Mendelian Randomisation (MR) analysis

Using GWAS summary data, we can investigate the causal factors of PAD by implementing MR method. Previous epidemiological studies have reported potential benefits of alcohol intake on various cardiovascular-related phenotypes, such as PAD. However, these observations may be biased by confounding and reverse causality. Conducting a Randomised Control Trial (RCT) requires a large effort and cost, which is not easy to conduct, whereas MR is an inbred RCT. MR analysis is possible on the following three assumptions: (1) the instrumental variable (SNP) is associated with the exposure with enough strength; (2) the instrumental variable (SNP) is associated with the outcome (disease) only through the exposure, i.e. there is no independent association between the SNPs and disease; (3) The instrumental variable must have no association with known or unknown confounders.

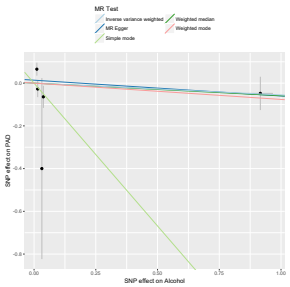
▪ Causal relationship between drinking and PAD

We selected five autosomal lead SNPs (rs1260326, rs1229984, rs3043, rs8187929 and rs671) associated with alcohol intake in Japanese population (Matoba N et al. *Nat Hum Behav* 2020) as instrument variables.

Using these SNPs, which would randomly assign subjects to drinker and nondrinker, we conducted MR analysis to infer the causal effect of drinking on PAD. The causal effect of drinking on PAD was negative and was not significant (Egger:

Beta (SE) = −0.07 (0.14), *P* 0.63; INW: Beta (SE) = −0.06 (0.12), *P* 0.63) (Figure 2).

Figure 2.

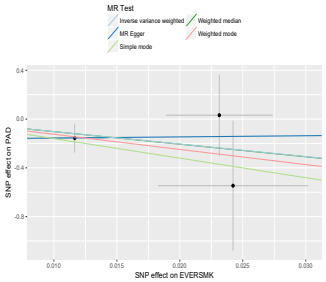


▪ Causal relationship between smoking and PAD

In order to test the causal relationship between smoking and PAD, we selected three SNPs (rs117036946, rs117097449 and rs77105140) that were reported to be associated with drinking status in Japanese (Matoba N et al. *Nat Hum Behav* 2019).

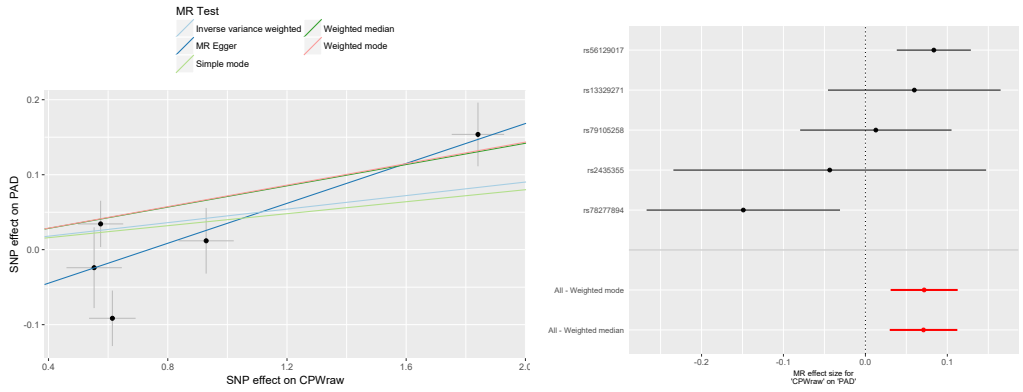
MR was conducted using these three SNPs as instrument variables. However, we found no role of smoking status in the development of PAD (Egger: Beta (SE) = −0.94 (26.1), *P* 0.98; INW: Beta (SE) = −10.2 (7.81) *P* 0.19) (Figure 3).

Figure 3.



We then selected five lead SNPs (rs78277894, rs2435355, rs79105258, rs13329271 and rs56129017) associated with the number of cigarettes smoked per day (Matoba N et al. *Nat Hum Behav* 2019). Using these five SNPs as instrument variables, which would randomly assign subjects to heavy-smoker and light-smoker, we conducted MR analysis to infer the causal effect of smoking on PAD. The slope was positive and the causal effect was significant (Egger: Beta (SE) = −0.07 (0.02), *P* 7.6×10^{-4}; INW: Beta (SE) = −0.07 (0.02) *P* 0.037) (Figure 4).

Figure 4.



4. Conclusions

We conducted GWAS for PAD and ran Mendelian Randomization analysis to infer the causal relationship between risk factors and development of PAD. Our large-scale single population GWAS detected novel and known loci associated with PAD in Japanese. We found no evidence of causal role of alcohol intake in the development of PAD. However, there was some evidence of causal role for smoking in increased risk of PAD.