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Shared and discrepant susceptibility for carotid artery and aortic arch calcification: A genetic association study

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ABSTRACT

Genome-wide association studies (GWASs) have identified several risk loci for coronary artery calcification. Four single-nucleotide polymorphisms (SNPs, rs1537370, rs1333049, rs2026458 and rs9349379) were associated with coronary artery calcification with *P* values less than 5×10^{-8} in GWASs. It is unclear if these associations exist in other vascular beds. Thus, we evaluated the impacts of these four SNPs on carotid artery and aortic arch calcification in this study. Computed tomography was applied to quantify the calcification of carotid artery and aortic arch. 860 patients with stroke completed calcification quantification and genotype testing were included in data analysis. Each SNP was evaluated for the association with carotid artery calcification, and with aortic arch calcification using generalized linear model. Among the four tested SNPs, rs2026458 was associated with calcification in both carotid artery ($\beta = 0.31$, 95% confidence interval [CI] 0.10–0.52, $P = 0.003$) and aortic arch ($\beta = 0.32$, 95% CI 0.10–0.54, $P = 0.004$), while rs1333049 was only associated with carotid artery calcification ($\beta = 0.28$, 95% CI 0.06–0.50, $P = 0.011$). In gender-stratified analyses, rs2026458 had significant impacts on carotid artery ($P = 0.003$) and aortic arch calcification ($P = 0.008$) in male, but not in female patients; while rs1537370 was significantly associated with carotid artery calcification in female ($P = 0.013$), but not in male patients. In conclusion, SNPs associated with coronary artery calcification may also increase the risk of calcification in other arteries such as carotid artery and aortic arch.

1. Introduction

Artery calcification is a dysregulation of matrix mineral metabolism involved in atherosclerotic lesions and is generally regarded as a quantitative index for the presence and progress of atherosclerosis [1,2]. The most frequently involved artery segments of atherosclerotic calcification include aorta, coronary, carotid and femoral arteries. Conspicuous artery calcification may cause hemodynamic abnormities in local lumen and increase the vulnerability of atherosclerotic plaque. Calcification, therefore, has been used as a predictor for the risk of myocardial infarction (MI) and ischemic stroke [3–5]. Concomitant calcifications in different vascular beds are common due to shared risk factors (hypertension, diabetic mellitus, dyslipidemia and smoking) and the systematical distribution of atherosclerosis [6,7]. However, the severity of atherosclerotic calcifications usually varies across vascular beds, and the calcifications often initiate sequentially, although sometimes overlap, in different arteries [8,9]. These observed phenomena indicate similar pathogenesis of, but dissimilar predisposition to atherosclerotic calcification in different vascular beds.

In recent years, genetic factors are increasingly regarded as a pathological basis for artery calcification. Genome-wide association

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studies (GWASs) have identified a number of single-nucleotide polymorphisms (SNPs) responsible for coronary artery calcification (CAC) [10–12]. Previous GWASs and other association studies on calcification mainly focused on coronary artery, and rarely involved in other vascular beds. Considering the universality of genetic effects as well as the varied structures of different arteries [13], we hypothesize that the same SNPs related to CAC may also influence calcifications in other arteries, but in different extent. To test this hypothesis, we evaluated the impacts of SNPs associated with CAC on calcifications in carotid artery and aortic arch in a cohort of Chinese patients with ischemic stroke.

2. Materials and methods

2.1. Study population

The study protocol was approved by the Ethical Review Board of Jinling Hospital. All participants were screened via Nanjing Stroke Registry Program (NSRP) between December 2009 and June 2013. NSRP has been described elsewhere in detail [14]. Informed consents were obtained from all participants.

Consecutive patients with acute ischemic stroke and aged 18 years or older were enrolled. All eligible patients underwent a neck computed tomography angiography (CTA) within 7 days after stroke onset. At the same day of CTA performance, venous blood was obtained and stored in −70 °C for genotyping. Exclusion criteria included: concurrent malignant neoplasms, severe liver or kidney diseases, parathyroid gland diseases, or calcium–phosphorus metabolism disorders. Considering that implanted stent may influence the accuracy of calcification assessment, 33 patients with history of carotid artery stenting and 2 patients with history of aortic arch stenting were also excluded. Finally, 878 patients were enrolled. Demographic characteristics and cardiovascular risk factors were collected and recorded, which included age, gender, histories of hypertension and diabetes mellitus (DM), smoking and drinking status and medications.

2.2. Measures of artery calcification

CTA was performed using a dual-source 64 slice CT system (Siemens, Forchheim, Germany) to quantify carotid artery calcification (CarAC) and aortic arch calcification (AorAC). Imaging was acquired by scanning from 4 cm below aortic arch to the superior border of orbit in the craniocaudal direction. Details of scanning parameters have been described previously [15]. Calcification scores in aortic arch and carotid artery were measured with commercially available software, Syngo Calcium Scoring (Siemens, Forchheim, Germany). A focus of $\geq$4 contiguous pixels accompanied by a CT density $\geq$130 Hounsfield units was defined as calcification according to the Agatston method [16]. The aortic arch was recognized as a section from the initial segment to the first centimeter of the common carotid arteries, the vertebral arteries, and the subclavian arteries beyond the origin of the vertebral arteries. As for the carotid artery, calcification was measured at both sides within 3 cm proximal and distal of the bifurcation including four artery segments: common, bulb, internal, and external. The reproducibility of calcification scores in the carotid artery and aortic arch was dual-assessed by two radiologists who were blinded to the genotypes.

2.3. Screening target SNPs

Two researchers (YZ, ZZ) searched Medline database for all GWASs on CAC-related SNPs published before December 31, 2013. With the search formula ((coronary artery) AND (calcification or calcified or calcium)) AND (genome-wide association), four SNPs from 2 GWASs [10,11] were identified based on the *P* values less than the significant threshold of 5×10^{-8} and a minor allele frequency (MAF) > 0.05 for Chinese Han population indexed in Hapmap Data Phase III (http://www.hapmap.org). Two of the four CAC-related SNPs, rs1333049 and rs1537370, are near the two cyclin-dependent kinase inhibitors, *CDKN2A* and *CDKN2B*. The other two SNPs, rs2026458 and rs9349379, are located in phosphatase and actin regulator 1 gene (*PHACTR1*).

2.4. DNA isolation and genotyping

Genomic DNA was extracted from whole blood with commercially available kits (TIANGEN Biotech (Beijing) Co., Ltd., Beijing). The 4 SNPs were genotyped using polymerase chain reaction ligase detection reaction (PCR-LDR) on an ABI Prism 377 Sequence Detection System (Applied Biosystems, Foster City, CA) [17], with technical support by Shanghai Genesky Biotechnology Company. Genotyping for every SNP was successfully operated for 99.7% samples. Reproducibility of genotyping was confirmed by a randomly selected 10% samples and the concordance was 100%.

2.5. Statistical analysis

Hardy–Weinberg equilibrium was calculated with Chi-square goodness-of-fit test or Fisher's exact test to examine the frequency distribution of each SNP between patients with and without artery calcification, linkage disequilibrium (LD) was analyzed with Haploview 4.2.

IBM SPSS Statistics Version 22.0 (Armonk, NY: IBM Corp.) was applied for statistical analyses. A threshold value less than 0.05 was considered statistically significant. Considering the extremely skewed distribution of calcification scores, we added 1 to each calcification score, and the value was then log-transformed with the following formula: Ln (calcification score + 1). This log-transformation may make the distribution of the parameter less skewed, as suggested by previous studies [10,11,18]. Linear regression was used to explore the association between each SNP and transformed calcification quantity. To test the accumulative effects of CAC-SNPs on artery calcification, we further constructed a genetic risk score (GRS) as previous study suggested [11]. Briefly, GRS for a given individual is derived by summing the number of risk alleles weighted by the effect estimate of the corresponding SNPs. In addition, we applied generalized multifactor dimensionality reduction (GMDR, http://sourceforge.net/projects/gmdr/) to identify the potential interactions of these four SNPs on degree of artery calcification. All analyses were performed with an additive model of inheritance and adjusted for age, gender, DM, hypertension, triglyceride, total cholesterol and smoking.

Power estimate was calculated with QUANTO Version 1.2.3 (http://biostats.usc.edu/software) for the linear regression analysis. By comparing with other models, we presumed that the additive genetic model is the fittest one for analyzing genetic effects on quantitative outcome of calcification in this sample with 860 patients. The effect size was explained as r^2 and estimated to be 0.01. When α was 0.05, the power estimate was 83.7% for analyzing association between SNPs and quantitative calcification.

3. Results

3.1. Baseline characteristics

Of the enrolled 878 patients, 15 cases failed in calcification scoring due to prominent calcification in nearby tissue, 3 cases failed in genotyping. Finally, 860 patients were included for data