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Genome-wide association study on progression of carotid artery intima media thickness over 10 years in a Chinese cohort

Gaoqiang Xie [a, *], Phyo Kyaw Myint [b, c, d, e], Deepak Voora [f], Daniel T. Laskowitz [g], Ping Shi [h], Fuxiu Ren [h], Hao Wang [i], Ying Yang [j], Yong Huo [j], Wei Gao [k, l], Yangfeng Wu [a, m, **]

[a] Peking University Clinical Research Institute, Beijing, China
[b] Epidemiology Group, Institute of Applied Health Sciences, School of Medicine and Dentistry, University of Aberdeen, Aberdeen, Scotland, United Kingdom
[c] Clinical Gerontology Unit, Department of Public Health and Primary Care, University of Cambridge, Cambridge, United Kingdom
[d] Norwich Research Park Cardiovascular Research Group, Norwich Medical School, University of East Anglia, Norwich, United Kingdom
[e] Stroke Research Group, Norfolk and Norwich University Hospital, Norwich, United Kingdom
[f] Duke University Genome Research Institute, USA
[g] Department of Neurology, Duke University Medicine Center, Durham, NC, USA
[h] Shijingshan Center for Disease Control and Prevention, Beijing, China
[i] State Key Laboratory of Cardiovascular Disease, Fuwai Hospital, National Center for Cardiovascular Diseases, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, 100037, China
[j] Department of Cardiology, Peking University First Hospital, Beijing, China
[k] Department of Cardiology, Peking University Third Hospital, Beijing, China
[l] Key Laboratory of Molecular Cardiovascular Sciences, Ministry of Education, Beijing, China
[m] Department of Epidemiology and Biostatistics, Peking University School of Public Health, Beijing, China

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ABSTRACT

Background: Carotid artery intima media thickness (IMT) in human is a marker of subclinical atherosclerosis with high heritability. Many genome-wide association studies (GWAS) were performed in European and American populations, yet discovery efforts have been limited in Asians.
Objective: To identify the genetic determinants of 10-year progression of IMT using GWA approach in a Chinese cohort.
Methods: Cardiovascular epidemiologic survey was carried out in 810 Chinese adults in 2012. 302,218 single-nucleotide polymorphisms (SNP) in whole genome were genotyped using gene chip and carotid IMT was measured. Most of these participants, had previous carotid IMT measurements in 2002 (n = 572), 2005 (n = 750), 2007 (n = 747), and 2010 (n = 671). General linear model (GLM) and multiple linear mixed-model (MLM) were used to assess the association between SNPs and carotid IMT.
Results: The mean age (SD) of the sample was 61.3 (5.1) years; 33.6% were men. The adjusted GLM showed no SNP with significance association at genome-level (all $p > 1 \times 10^{-7}$). However, using MLM, after adjusting for age, sex, number of cigarettes smoked per day, systolic blood pressure, use of antihypertensive drugs in the past 2 weeks, serum cholesterol, body mass index, fasting glucose levels, use of insulin or hypoglycemic drugs, time of measuring IMT and its interaction with SNP, we identified two novel SNPs (rs36071027 in EBF1 gene on chromosome 5 and rs975809 close to PCDH15 gene on chromosome 10) that are significantly associated with carotid IMT at genome level ($p < 1 \times 10^{-7}$) and seven novel SNPs (rs2230307 in AGL gene on chromosome 1, rs12040273 in GALNT2 gene on chromosome 1, rs4536103 in NEUROG3 gene on chromosome 10, rs9855415 in LOC647323 gene on chromosome 3, rs2472647 in PCDHGA1 gene on chromosome 5, rs17433780 in GBP3 gene on chromosome 1, and rs7625806 in DLEC1 gene on chromosome 3) which are suggestive of significant association ($p < 10^{-5}$).

* Corresponding author. Room 205, No. 7 Building, Xue Yuan Road 38#, Haidian District, Beijing, 100191, China.
** Corresponding author. No. 6 Building, Xue Yuan Road 38#, Haidian District, Beijing, 100191, China.
E-mail addresses: gxie@bjmu.edu.cn (G. Xie), wuyf@bjmu.edu.cn (Y. Wu).

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Conclusion: The study represents the first GWAS of association between SNPs and carotid IMT in an Asian population. We identified 2 novel loci associated with carotid IMT progression over 10 years.

1. Introduction

Subclinical atherosclerosis is a chronic condition with increased risk of cardiovascular and cerebrovascular diseases which are leading causes of mortality and morbidity worldwide. Unlike single gene disorders, its pathogenesis is complex and involves multiple risk factors including genetic and non-genetic factors, and also influenced by their interactions [1].

As a marker of pre-symptomatic atherosclerosis, carotid intima-media thickness (IMT) has been demonstrated to correlate well with risk of ischaemic cerebrovascular and cardiovascular events [2,3]. In Chinese population, which constitutes almost a quarter of the world's population, the prevalence of carotid plaque is common and reported at 36.9% (47.2% for adult men, 31.3% for adult women) [4]. These alarming figures highlight the need for deeper insight and better understanding of the aetiology and pathogenesis of early atherosclerosis in Chinese population if we are to decrease the global burden of CVD.

Numerous studies have previously reported consistent evidence of moderate heritability of carotid IMT [5,6]. A large meta-analysis of genome-wide association study shows that there are several significant SNPs for IMT in European and American populations [7]. In Asians, a candidate gene study suggested that the differences of IMT among individuals may be attributed to genetic variants, such as rs4803455 in transforming growth factor-β1(TGFB1) gene ($p = 0.03$) [8]. However, previously we found that rs1800872 of interleukin-10 gene was not associated with carotid IMT [9]. Moreover, to date no Genome-wide association studies (GWAS) in Asian populations have been carried out.

We therefore performed a GWAS on progression of carotid IMT in a community sample from Beijing, China over a 10-year follow-up.

2. Methods

2.1. Study population

The study population consisted of participants from rural Beijing and they were drawn from the original cohort of the People's Republic of China–United States of America (PRC–USA) Collaborative Study of Cardiovascular and Cardiopulmonary Epidemiology. A detailed description of the goals, design and methods of the PRC–USA study has been published elsewhere [10,11]. Briefly, genome-wide 302,218 SNPs were genotyped in 810 Chinese adults aged 53–79 years old without stroke, myocardial infarction, and cardiovascular surgery in 2012. At the same time, carotid artery intima media thickness (IMT) was measured using carotid ultrasound. Most of them had IMT measured in preceding years: 2002 ($n = 572$), 2005 ($n = 750$), 2007 ($n = 747$), and 2010 ($n = 671$). A total of 442 subjects (54.57%) had carotid IMT measurements at all time points (with a total of five carotid IMT measurements over a 10-year period) (see Supplement A in file S1).

The Cardiovascular Institute and Fuwai Hospital Ethics Committee approved the 2002, 2005, and 2010 surveys, and the Peking University Health Science Center Ethics Committee approved the 2007 and 2012 surveys. Informed written consent was obtained from all participants in all surveys and examinations.

2.2. Genotyping and quality control

Lymphocytic DNA samples were obtained from all participants and Illumina HumanExome BeadChip (Illumina, Inc. San Diego, CA 92122 USA) was used for genotyping which provided excellent coverage of all common genetic variations. The positions and corresponding genes of these SNPs were identified by the National Center for Biotechnology Information map viewer (www.ncbi.nlm.nih.gov/mapview/) and HumanExome BeadChip annotation data. Among 302,218 SNPs genotyped, we excluded SNPs with MAF < 0.10 ($n = 265,071$), those with genotype call rate <95% ($n = 933$); or deviation from Hardy–Weinberg equilibrium (P value < 0.05; $n = 2404$), and unsatisfactory genotypic quality (genotypic quality score <0.25, $n = 995$) (see Supplement B in file S1). Finally, 810 samples and 32,817 SNPs were entered into the subsequent analysis.

2.3. Ultrasound protocol

The same protocol was used for all carotid IMT measurements in all five examinations and the protocol has been published elsewhere [4,9]. In brief, carotid IMT was measured on both sides at the far wall of three 10-mm segments of carotid artery at (1) the proximal common carotid artery, (2) the distal common carotid artery, and (3) the bifurcation. In every segment, measurements were attempted at three points (0, 5 and 10 mm), provided these points were free of plaque. The overall value of the IMT was calculated as average of 18 measurements ($2 \times 3 \times 3$) at each time points.

Increase in IMT was calculated using the following formula:

$$\mathrm{IMT_{inc}} = \frac{\sum_{i=1}^{n}(x_i - \overline{x})(y_i - \overline{y})}{\sum_{i=1}^{n}(x_i - \overline{x})^2}$$

where x_i = year of measuring IMT, $y_i = \mathrm{IMT_{year}}$.

All IMT measurements had good reproducibility. The baseline IMT measurements in 2002 using the ACUSON-ASPEN (Acuson Inc., Mountain View, California, USA) by trained sonographers have been shown to have high reproducibility [4]. A reproducibility study was conducted in 20 subjects by performing two additional scans. The inter-observer coefficient of variation between the two observers was 4.42%. The intra-observer variability, described as the mean of the absolute difference between the first and second observation, was 0.037 mm. A paired t-test showed no statistically significant difference between the 2 measures [4]. The subsequent IMT measurements in 2005, 2007, 2010, and 2012 were performed according to the same method as baseline in 2002. Among 442 participants with a total of five carotid IMT measurements, the IMT in 2002 showed significant correlation with the IMT measured in subsequent years; 2005 ($r = 0.41$, $p < 0.001$), 2007 ($r = 0.536$, $p < 0.001$), 2010 ($r = 0.471$, $p < 0.001$), and 2012 ($r = 0.521$, $p < 0.001$). The Cronbach's Alpha of those five IMT measurements was 0.75 indicating good reliability of these measurements.

2.4. Baseline risk factor measurement

At the baseline, all major conventional cardiovascular risk