Atherosclerosis 226 (2013) 433–439

Contents lists available at SciVerse ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

The M235T single nucleotide polymorphism in the angiotensinogen gene is associated with coronary artery calcium in patients with a family history of coronary artery disease☆

Parag H. Joshi [a,b], Hongyan Xu [c], Renee LeStrange [b], Nancy Flockhart [b], Ben Kirkland [b], Gustavo Vazquez [b], Zhen Qian [b], Abhinav Sharma [b], Idean Marvasty [b], Kunal Bhatt [b], Charles Brown [b], Sarah Rinehart [b], Joseph Miller [b], Szilard Voros [d,e,*]

[a] Johns Hopkins University School of Medicine, Baltimore, MD, USA
[b] Piedmont Heart Institute, Atlanta, GA, USA
[c] Georgia Health Sciences University, Augusta, GA, USA
[d] Stony Brook University Medical Center, Stony Brook, NY, USA
[e] Global Genomics Group, Richmond, VA, USA

ARTICLE INFO

Article history:
Received 22 May 2012
Received in revised form
21 September 2012
Accepted 9 October 2012
Available online 16 October 2012

Keywords:
Coronary artery calcification
Family history
Candidate genes
Angiotensinogen

ABSTRACT

Little is known about the contribution of genetics and lipoprotein subclasses to the development of coronary artery calcification (CAC) in asymptomatic, first-degree relatives of patients with CAD. We evaluated 100 asymptomatic, non-smoking, lipid-lowering therapy-naïve, first-degree relatives of patients with obstructive CAD through testing for 27 biomarkers, 15 single nucleotide polymorphisms in 12 candidate genes, and CAC and compared them to matched controls without family history. We compared prevalence of CAC in those with and without family history and biomarkers between those with and without CAC. Mean age was 41.6 ± 9 years; 58% were female. Significantly more subjects with family history had non-zero CAC (median Agatston: 13, range 1–1107) compared to those without family history (median Agatston: 43; range 1–345) (21% vs. 9%; $p = 0.028$). Among subjects with family history, in subjects with positive vs. negative CAC, multivariable analysis showed significantly lower HDL-2A (999 ± 333 vs. 1262 ± 397 nmol/L) and higher frequency of a substitution of threonine for methionine at codon 235 in the angiotensinogen gene (AGT M235T) (75% vs. 54%; $p < 0.05$; odds ratio of 2.6 for CAC). Population attributable risk of one copy of the risk allele at the AGT locus was 16%, highest of any variable tested. In conclusion, in this population of healthy, low-risk subjects with a family history of CAD, the AGT M235T variant was the most significant predictor of CAC independent of blood pressure, raising the possibility of an alternative biological pathway.

1. Introduction

Both genetic and environmental factors contribute to the development of atherosclerosis. Some single nucleotide polymorphisms (SNPs) have been directly linked to coronary artery disease (CAD) (e.g. 9p21 [rs1333049]) [1] while others have been linked to intermediate phenotypes, such as dyslipidemia and hypertension. The angiotensinogen (AGT) SNP, M235T (rs699), which results in a change from methionine to threonine at the 235th amino acid position, has been linked to hypertension in several studies and to coronary artery disease (CAD) in some studies, defined by myocardial infarction, unstable angina, or angiographic evidence of CAD requiring intervention [2,3]. Angiotensinogen, produced in the liver, is a key component of the renin–angiotensin system (RAS) and regulates blood pressure through maintenance of blood volume, vessel constriction and electrolyte balance. In addition to this classical model, considerable evidence points toward an association between the RAS and atherosclerosis [4]. The exact change in function of the resulting angiotensinogen protein from the M235T SNP is unknown and there is conflicting evidence regarding the resulting circulating levels of angiotensinogen as well as the association with atherosclerosis [5,6].

☆ Work was performed at Piedmont Heart Institute, Atlanta, GA, USA (for affiliations "Johns Hopkins University School of Medicine", "Stony Brook University Medical Center" and "Global Genomics Group").

* Corresponding author. Stony Brook Medicine, HSC 4, Room 120, Stony Brook, NY 11794-8460, USA. Tel.: +1 631 444 1011; fax: +1 631 444 7538.
E-mail address: szilardvorosmd@gmail.com (S. Voros).

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http://dx.doi.org/10.1016/j.atherosclerosis.2012.10.039

Coronary artery calcium (CAC) on non-contrast cardiac computed tomography (CT) is highly specific for the presence of atherosclerosis and may be a more appropriate phenotype compared to traditional phenotypes such as the history of CAD, MI or even invasive angiography.

The contribution of common variants in candidate genes, such as the angiotensinogen M235T variant, to atherosclerosis is unknown. Thus, we hypothesized that SNPs associated with intermediate phenotypes including blood pressure, cholesterol metabolism, and inflammation contribute to atherosclerosis as measured by CAC. Accordingly, this study was designed to compare the prevalence of CAC in subjects with a family history of CAD against age and gender-matched controls without a family history of CAD and to assess the contribution of known SNPs in pre-specified candidate genes involved in intermediate phenotypes.

2. Methods

This was a prospective, single center, observational study approved by the Institutional Review Board of Piedmont Healthcare (Atlanta, Georgia). All subjects provided written, informed consent.

2.1. Study design

Fifty probands were recruited with a history of obstructive CAD diagnosed at any age (defined as 70% diameter stenosis in a major epicardial vessel or any prior revascularization). For each proband, two first-degree relatives (parents, children, or siblings) were recruited if they were clinically asymptomatic, non-smokers, non-diabetic, lipid-lowering therapy-naive, and without known coronary atherosclerosis. We excluded patients with environmental risk factors and intermediate phenotypes that may lead to potential confounding of genetic data (Supplementary Table). Symptoms were obtained per the Diamond–Forrester Criteria [7]. Smoking status was obtained by self-report of tobacco use in the previous 10 years. Diabetic status and diabetic medication use was also obtained by self-report. This resulted in 50 probands and 100 family members for a total of 150 subjects who underwent testing of intermediate phenotypes including comprehensive biomarker testing and calcium scoring.

In order to assess the contribution of family history to CAC, we performed a case: control study by matching each family member in a 1:1 ratio based on age and gender from a population of asymptomatic, non-diabetic, non-smoking subjects without any family history of CAD who were referred for calcium scoring; age was matched within 3 years for each participant. Calcium scores and MESA percentile were recorded.

2.2. Clinical data

Symptoms, past medical history, social history, family history, current and past medications, vital signs, height, weight, and waist circumference were obtained by research personnel. Whole blood, serum, plasma, and buffy-coat samples were collected and stored at −80 °C. Framingham risk score was calculated using the publicly available online calculator from the National Heart, Blood, and Lung Institute website (http://hp2010.nhlbihin.net/atpiii/calculator.asp?usertype=pub).

2.3. Genotype testing

Genotype testing was performed using a commercially available panel (Genovations, Genova Diagnostics; Asheville, North Carolina), which included the AGT M235T SNP and 14 other SNPs with possible relevance to intermediate phenotypes related to cardiovascular disease (Table 1). Using a solution-hybridization method, in which two oligonucleotides hybridize in tandem with specific DNA sequences with subsequent generation of a fluorescent signal, the polymorphisms are then detectable with high accuracy [8].

2.4. Lipoprotein and biomarker testing

Fasting lipid profile, including total cholesterol (TC), triglycerides (TG), and high- and low-density lipoprotein cholesterol (HDL-C, LDL-C) was performed by standard enzymatic methods. Additionally, samples underwent lipoprotein fractionation by ion mobility through a commercially available laboratory (Quest Diagnostics; Tempe, Arizona). This method provides LDL particle number, LDL particle size, LDL subfractions (I–IV), HDL subfractions (2B, 2A, 3), intermediate density lipoprotein subfractions (IDL 1–2), and very low-density lipoprotein subfractions (VLDL large, intermediate, and small). Additionally, apoprotein B, lipoprotein (a) [Lp(a)], high-sensitivity C-reactive protein (hsCRP), homocysteine, and insulin were measured (Quest Diagnostics; Tempe, Arizona). Apoprotein AI was measured by nephelometry and rate turbidimetry at the Piedmont Heart Institute. Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) was measured by immunoturbidimetry through a commercially available laboratory (Berkeley Heart Lab; San Francisco, California).

2.5. Coronary artery calcium scanning

CAC scanning was performed using either a 32 × 2 multi-slice computed tomography system (Siemens Somatom 64; Erlangen, Germany) or a 320-detector row system (Toshiba Aquilion ONE; Tustin, CA). On the 32 × 2-detector row platform, CAC was obtained with non-contrast enhanced scans using 3.0 mm collimation with 2.0 mm interslice gap. Acquisition parameters included a gantry rotation of 330 ms, pitch 0.24, tube voltage 120 kV and tube current of 250 mA. On the 320-detector row platform, CAC was obtained with a prospectively triggered, single-beat, volumetric protocol. CAC was quantified using Agatston's method [9]. Additionally, using the publicly available online calculator from the Multi Ethnic Study of Atherosclerosis (MESA) study, a ranking percentile was calculated for calcium scores for a subject's age, ethnicity, and gender [10].

2.6. Statistical analysis

Normally distributed continuous variables are expressed as mean ± SD and compared by student's *t*-test while non-normally distributed variables are expressed as medians with interquartile range (25th and 75th percentiles) and compared by Kruskal–Wallis analysis. Genotype distributions were tested with the expected proportions under Hardy-Weinberg Equilibrium (HWE) using goodness-of-fit chi-square tests. Chi-square and Fisher's exact tests were performed to test the association of genotypes at the candidate SNP loci and presence of CAC using information from unrelated family members. To account for family correlations, a mixed-model logistic regression approach was utilized to identify SNPs associated with CAC with adjustment for age, ethnicity, and gender [11]. Significant variables from unadjusted models were included in the final, multivariable regression model. The population attributable risk was estimated using the odds ratio estimate obtained from the logistic regression model as previously described [11]. All analyses were performed using R 2.11 (http://www.r-project.org) and SAS 9.2 (Cary, NC).