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Variation in *ANGPTL4* and risk of coronary heart disease: the Atherosclerosis Risk in Communities Study

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Abstract

An E40K loss-of-function variant in the *ANGPTL4* gene is associated with substantially reduced plasma triglyceride levels in white persons, but its association with cardiovascular disease occurrence has not been reported. The prospective, population-based Atherosclerosis Risk in Communities Study measured the E40K *ANGPTL4* variant in approximately 10000 white participants and determined its association with coronary heart disease (CHD) incidence (n = 1318 events) between 1987-1989 and 2004. Compared with noncarriers, carriers of 1 or 2 copies of the 40K variant (3.8% frequency) had a 19-mg/dL lower age- and sex-adjusted mean triglyceride level, 5-mg/dL lower low-density lipoprotein cholesterol, and 4-mg/dL higher high-density lipoprotein cholesterol. The age-, sex-, and field center–adjusted hazard ratio of CHD was 0.63 (95% confidence interval, 0.45-0.89). Adjustment for nonlipid confounding factors did not change this hazard ratio appreciably. Carriers also appeared to have reduced risk of incident stroke, prevalent peripheral artery disease, and carotid atherosclerosis; but these associations were based on few events among 40K carriers and were not statistically significant. In conclusion, in this prospective study, the 40K variant of *ANGPTL4* appeared to confer reduced genetic risk for CHD.

1. Introduction

It is well established that higher blood levels of low-density lipoprotein cholesterol (LDL-C) and lower levels of high-density lipoprotein cholesterol (HDL-C) increase the risk of atherosclerotic cardiovascular disease (CVD). Whether higher blood triglyceride levels increase the risk of atherosclerotic events has been less clear. However, a recent meta-analysis involving 10158 incident coronary heart disease (CHD) cases from 29 studies and corrected for within-person measurement error reported that triglycerides have a moderate independent association with CHD incidence [1]. Comparing the top vs the bottom third of usual log-triglyceride values, the adjusted odds ratio (OR) of CHD was 1.72 (95% confidence interval [CI], 1.56-1.90).

A recent report from 3 cohort studies indicated that an E40K loss-of-function variant in *ANGPTL4*, a gene involved in partitioning of fatty acids between sites of storage and sites of oxidation, is associated with substantially reduced plasma levels of triglyceride and increased HDL-C in white persons [2]. The E40K polymorphism, which is not currently listed in the Single Nucleotide Polymorphism Database, entails a 118G to A base substitution at codon 40 changing the amino acid from glutamic acid to lysine. In the Atherosclerosis Risk in Communities (ARIC) Study, the 40K variant (1 or 2 copies) was present in 4% of white persons but was very rare in African Americans. Besides lower triglyceride and higher HDL-C, 40K carriers had modestly decreased LDL-C and insulin levels [2]. Whether the *ANGPTL4* E40K variant is associated with cardiovascular events has not yet been explored.

We used data from the ARIC Study to determine whether there is an association between the *ANGPTL4* E40K variant and incidence of CHD. As secondary analyses, some of which had low statistical power, we

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also looked at associations with prevalence of carotid atherosclerosis or peripheral artery disease (PAD) and incidence of ischemic stroke.

2. Materials and methods

2.1. Population

The ARIC Study is a cohort study of CVD in 4 US communities [3]. Between 1987 and 1989, 7082 men and 8710 women aged 45 to 64 years were recruited from Forsyth County, North Carolina; Jackson, MS (African Americans only); suburban Minneapolis, MN; and Washington County, Maryland. The ARIC Study protocol was approved by the institutional review board of each participating university. After written informed consent was obtained, including that for genetic studies, participants underwent a baseline clinical examination (visit 1). Follow-up examinations of the cohort occurred 3 times, at intervals of roughly 3 years. The response rates for visits 2 (1990-1992), 3 (1993-1995), and 4 (1996-1998) were 93%, 86%, and 80%, respectively. Participants completed annual telephone interviews between visits and after visit 4.

2.2. Risk factor measurements

Risk factors examined in these analyses were ascertained at visit 1, as described in detail in the ARIC Study manuals of operation [4]. Participants were asked to fast for at least 12 hours before the clinical examination. Blood was drawn from an antecubital vein of seated participants into vacuum tubes containing ethylenediaminetetraacetic acid (for measurement of lipids and DNA extraction) or a serum separator gel (glucose). Serum and plasma aliquots were stored at −70°C and were shipped to central laboratories for analyses. Total cholesterol and triglycerides were measured by enzymatic methods, and HDL-C was measured after dextran-magnesium precipitation. Low-density lipoprotein cholesterol was calculated [5]. Serum glucose was assayed by a hexokinase/glucose-6-phosphate dehydrogenase method. *Prevalent diabetes mellitus* was defined as a fasting glucose of at least 126 mg/dL [6] or a self-reported history of or treatment of diabetes. Seated systolic and diastolic blood pressures (SBP and DBP) were measured 3 times using a random-zero sphygmomanometer, and the average of the last 2 measurements was used for analysis. A standard 12-lead electrocardiogram was recorded.

Anthropometrics were taken with the subject wearing a scrub suit and no shoes. Body mass index (BMI) was calculated (weight in kilograms/height in meters squared). Questionnaires assessed education; smoking status; antihypertensive and lipid-lowering medications within the past 2 weeks; number of cigarettes smoked per day and duration of smoking (pack-years computed); and usual consumption of wine, beer, and hard liquor (grams per day computed). Level of sports physical activity was assessed by the questionnaire of Baecke et al [7].

2.3. Genotyping

The E40K single nucleotide polymorphism is located at position 1033320 in Contig NT_077812.2 (National Center for Biotechnology Information genome build 36.2), and context sequence is as follows:

GTCGCCGCGCTTTGCGTCCTGGGAC[G/A]AGATGAATGTCCTGGCGCACGGACT.

Using stored DNA from ARIC participants, fluorogenic 5′-nucleotidase assays for the *ANGPTL4* alleles encoding E40K or the wild-type protein were performed using the TaqMan assay system (Applied Biosystems, Foster City, CA), as previously described [2]. The assays were carried out on a 7900HT Fast Real-Time PCR instrument with probes and reagents purchased from Applied Biosystems.

2.4. Ascertainment of prevalent CVD

For exclusion in incidence analyses, *prevalent CHD at baseline* was defined as a self-reported history of physician-diagnosed myocardial infarction (MI), coronary artery bypass surgery, or coronary angioplasty, or evidence of a previous MI by electrocardiogram. *Prevalent stroke* was defined, for exclusion, as a self-reported history of physician-diagnosed stroke.

Prevalent PAD was defined as intermittent claudication by the questionnaire of Rose et al [8] or an ankle/brachial BP index less than 0.9. The ankle/brachial BP index was computed by dividing the average of ankle SBP measurements by the average of brachial SBP measurements [7]. Using the Dinamap 1846 SX automated oscillometric device (Criticon, Tampa, FL), trained technicians measured 2 ankle BPs, taken 5 to 8 minutes apart, at the posterior tibial artery in a randomly selected leg while the participant was prone. This automated BP measurement device has high validity compared with the standard Doppler ultrasound measurement and high reliability [9]. Two brachial artery SBPs were measured, usually in the right arm, with the participant supine [10]. The questionnaire of Rose et al identifies intermittent claudication as exertional leg pain relieved within 10 minutes by resting.

High-resolution B-mode ultrasound (Biosound 2000 II SA; Biosound, Indianapolis, IN) was used to measure intima-media thickness (IMT) bilaterally in the extracranial carotid arteries, in the areas of the common carotid artery (1 cm proximal to the dilatation of the carotid bulb), the carotid bifurcation (1 cm proximal to the flow divider), and the internal carotid artery (1 cm distal to the flow divider). Standardized protocols for scanning and reading were used based on a technique validated by Pignoli et al [11]. To enhance the reproducibility of carotid artery measurements, standardized interrogation angles were used. Centralized training, certification, and quality control programs were implemented for both the sonographers and the readers to ensure reliability and validity of these measurements [12]. The mean IMT values at the 6 carotid sites were combined to produce an overall mean IMT. In case of missing data at any