Atherosclerosis 241 (2015) 641–648

Contents lists available at ScienceDirect

Atherosclerosis

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

Lipoprotein associated phospholipase A_2 activity, apolipoprotein C3 loss-of-function variants and cardiovascular disease: The Atherosclerosis Risk In Communities Study

Yashashwi Pokharel [a, b, 1, *], Wensheng Sun [a], Linda M. Polfus [c], Aaron R. Folsom [d], Gerardo Heiss [e], A. Richey Sharrett [f], Eric Boerwinkle [c, g], Christie M. Ballantyne [a, b], Ron C. Hoogeveen [a, b]

[a] Section of Cardiovascular Research, Department of Medicine, Baylor College of Medicine, Houston, TX, USA
[b] Center for Cardiovascular Disease Prevention, Methodist DeBakey Heart and Vascular Center, Houston, TX, USA
[c] University of Texas Health Science Center at Houston, Houston, TX, USA
[d] School of Public Health, Division of Epidemiology and Community Health, University of Minnesota, Minneapolis, MN, USA
[e] Gillings School of Global Public Health, The University of North Carolina at Chapel Hill, Chapel Hill, NC, USA
[f] Bloomberg School of Public Health, Department of Epidemiology, Johns Hopkins University, Baltimore, MD, USA
[g] Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX, USA

ARTICLE INFO

Article history:
Received 16 April 2015
Received in revised form
5 June 2015
Accepted 16 June 2015
Available online 18 June 2015

Keywords:
Apolipoprotein C3 loss-of-function
Atherogenic dyslipidemia
Atherosclerosis Risk In Communities Study
Cardiovascular disease
Coronary heart disease
Ischemic stroke
Lipoprotein-associated phospholipase A_2 activity

ABSTRACT

Objective: Lipoprotein-associated phospholipase A_2 ($LpPLA_2$) activity was associated with higher CHD risk in a meta-analysis, which was partly dependent on circulating lipid levels. Apolipoprotein C3 loss-of-function (ApoC3 LOF) mutations were related with reduced postprandial lipemia and CHD risk. However, the association of $LpPLA_2$ activity with ApoC3 LOF is not known.

Methods: We examined the association of $LpPLA_2$ activity and ApoC3 LOF mutations and incident cardiovascular disease (CVD) (defined as coronary heart disease [CHD] plus ischemic stroke) and all-cause mortality in the biracial longitudinal Atherosclerosis Risk In Communities (ARIC) study.

Results: The mean $LpPLA_2$ activity was 229.3 nmol/min/mL and was higher in men and whites. $LpPLA_2$ activity correlated positively with atherogenic dyslipidemia. ApoC3 LOF carriers had lower $LpPLA_2$ activity levels compared to non-carriers, and there was inverse association between $LpPLA_2$ activity and ApoC3 LOF mutations in whites. In a fully adjusted model, greater $LpPLA_2$ activity was independently associated with incident CVD (HR 1.35, 1.09−1.68 for highest vs. lowest quintile), which was mainly explained by its association with CHD, and was also associated with all-cause mortality (HR 1.65, 1.38 −1.98).

Conclusions: Greater $LpPLA_2$ activity was associated with increased CHD and all-cause mortality in both whites and African−Americans in the ARIC study. The inverse relation between $LpPLA_2$ activity and ApoC3 LOF mutations suggests that delayed lipoprotein clearance may at least in part explain the observed association of $LpPLA_2$ activity with increased CVD risk.

Abbreviations: ApoB, apolipoprotein B; ApoC3 LOF, apolipoprotein C3 loss-of-function; ARIC, Atherosclerosis Risk In Communities; CHD, coronary heart disease; CVD, cardiovascular disease; HDL-C, high-density lipoprotein cholesterol; LDL(C), low-density lipoprotein (cholesterol); $LpPLA_2$, lipoprotein-associated phospholipase A_2; PLA2G7, Phospholipase A_2, Group VII (Platelet-Activating Factor Acetylhydrolase, Plasma); PtdCho, phosphatidylcholine; sdLDL-C, small dense low-density lipoprotein cholesterol; SOLID-TIMI 52, the Stabilization of Plaques using Darapladib; STABILITY, the Stabilization of Atherosclerotic Plaque by Initiation of Darapladib Therapy.

* Corresponding author. 6565 Fannin St. Suite B 157, Section of Cardiovascular Research, Baylor College of Medicine, Houston, TX 77054, USA.
E-mail addresses: yashashwi.pokharel@bcm.edu, ypokharel@saint-lukes.org (Y. Pokharel), wsun@bcm.edu (W. Sun), Linda.M.Whitaker@uth.tmc.edu (L.M. Polfus), folso001@umn.edu (A.R. Folsom), gerardo_heiss@unc.edu (G. Heiss), rsharret@jhu.edu (A.R. Sharrett), Eric.Boerwinkle@uth.tmc.edu (E. Boerwinkle), cmb@bcm.edu (C.M. Ballantyne), ronh@bcm.edu (R.C. Hoogeveen).
[1] Starting July 1, 2015 Mid-America Heart Institute, University of Missouri-Kansas City, 4401 Wornall Road, SLNI, CV Research 5th Floor, Kansas City, MO 64111, USA

http://dx.doi.org/10.1016/j.atherosclerosis.2015.06.033
0021-9150/

1. Introduction

The concept that atherosclerosis is an inflammatory disease is supported by both the presence of inflammatory cells in the cap of atherosclerotic plaques and reports that elevated inflammatory markers in circulation are associated with increased incidence of coronary heart disease (CHD) [1]. The oxidative modification of low-density lipoproteins (LDL) within the arterial wall is a key early event in the development of atherosclerosis [2]. It involves the oxidation of polyunsaturated fatty acid component of phospholipids and ultimately leads to the conversion of phosphatidylcholine (PtdCho) to lyso-PtdCho [3]. The increased lyso-PtdCho content of oxidized LDL is a chemoattractant for human monocytes and induces endothelial dysfunction [4,5]. Lipoprotein-associated phospholipase A_2 ($LpPLA_2$) is a serine-dependent lipase that has been shown to hydrolyze oxidatively modified PtdCho to release oxidized fatty acids and lyso-PtdCho [6]. $LpPLA_2$ is secreted by inflammatory cells in atherosclerotic plaques [7] and is primarily responsible for the phospholipase activity associated with LDL [8]. The expression of $LpPLA_2$ is regulated by inflammatory mediators and inhibition of $LpPLA_2$ activity results in decrease in both lyso-PtdCho content and monocyte chemoattractant ability of oxidized LDL [8,9]. Recently the food and drug administration approved $LpPLA_2$ activity to predict CHD risk [10].

A meta-analysis by the $LpPLA_2$ Studies Collaboration showed that higher $LpPLA_2$ activity portends increased CHD risk but not ischemic stroke over a median of 6 years of follow-up [11]. However, the results from the individual studies included in the meta-analysis were not consistent. Furthermore, in subgroup analysis the association with CHD was significant only in individuals with stable CHD, but not in those without history of CHD. Two large clinical trials designed to lower $LpPLA_2$ activity using darapladip did not lower cardiovascular events in patients with established CHD [12,13]. A recent study showed that variations in Phospholipase A2, Group VII gene (*PLA2G7)* that reduce $LpPLA_2$ activity, did not have any effect on CHD risk [14].

Apolipoprotein C3 (ApoC3) has been shown to inhibit the lipolytic activity of lipoprotein lipase and can promote delayed clearance of atherogenic lipoproteins [15]. Furthermore, ApoC3 loss-of-function (LOF) variants are associated with lower triglycerides and small dense low-density lipoprotein cholesterol (sdLDL-C) levels and higher high-density lipoprotein cholesterol (HDL-C) levels, reduced postprandial lipemia and reduced CHD risk [16]. Therefore, it is possible that individuals with ApoC3 LOF variants have lower plasma $LpPLA_2$ activity levels due to lower levels of circulating atherogenic particles. In the Atherosclerosis Risk In Communities (ARIC) study, we previously studied the associations of $LpPLA_2$ mass with CHD and ischemic stroke using a case cohort design [17,18]. In the current study we investigated the relationship of $LpPLA_2$ activity with ApoC3 LOF variants and the risk for incident cardiovascular disease (CVD) in whites and African–Americans in the ARIC study.

2. Material and methods

2.1. Study population

The ARIC study is a prospective epidemiologic study of 15,792 participants initially between 45 and 64 years of age from 4 U.S. communities started in 1987. Detailed information on the study design, objectives and sampling strategy has been previously described [19]. ARIC cohort visit 4 conducted between 1996 and 1998, consisting of 11,656 participants, served as the baseline for the present analysis. After excluding individuals with races other than African Americans or whites (n = 31); African American participants from the Minnesota or Washington County field centers (n = 37) (because their numbers were too small to provide good estimates for their race/center combinations); and 416 subjects without information on $LpPLA_2$ and other covariates, a total of 11,172 participants aged 54–74 years were available for our analysis. For each outcome of interest, individuals with prevalent disease were excluded. For example, with coronary heart disease (CHD) as an outcome, individuals with prevalent CHD were excluded, and with ischemic stroke as an outcome, individuals with prevalent ischemic stroke were excluded. Follow up time ended when the participant had an outcome, died, was lost to follow-up, or survived until December 31st 2009.

2.2. Covariates

Medical history, demographic data, anthropometric data, blood pressure measurements and fasting lipids were obtained during visit 4 following a standardized protocol. Participants were asked to fast for 12 h before the clinic visit and 86% reported doing so. Diabetes was defined as fasting blood glucose level ≥126 mg/dL, non-fasting blood glucose level ≥200 mg/dL, or self-reported physician diagnosis of or treatment for diabetes. Total cholesterol and high-density lipoprotein cholesterol (HDL-C) were determined by enzymatic methods [20]. Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation [21], sdLDL-C was measured using a novel homogeneous assay [22], and plasma apolipoproteins and high-sensitivity C-reactive protein were measured by immunonephelometric assay [22]. Plasma $LpPLA_2$ activity was measured in samples stored for approximately 10 years at −70 °C using an automated Colorimetric Activity Method assay (diaDexus Inc., South San Francisco, CA) using a Beckman Coulter (Olympus) AU400e autoanalyzer. The $LpPLA_2$ activity assay had an inter-assay variation coefficient of 4.4% and a reliability coefficient of 0.92, based on 419-blinded replicate samples.

DNA sequencing was performed on Illumina HiSeqs (San Diego, CA) after exome capture with NimbleGen's VCRome2.1. Prior to statistical analysis, data were processed and alleles jointly called using Mercury [23].

Many of the same ApoC3 variants reported previously were identified in our study. Three variants were identified in 23 carriers among white participants. These were 11:116701353 (rs76353203, **R19X**, nonsense, 3 carriers), 11:116701354 (rs138326449, **IVS2+1G->A**, splice, 19 carriers) and 11:116701613 (rs140621530, **IVS3+1G->T**, splice, 1 carrier). Two of these three variants (11:116701353 and 11:116701613) were validated through genotyping from the exome chip array and exhibited 100% concordance with the overlapping ARIC participant's exome sequence variant. The 4 variants identified in 11 carriers in African–American participants were 11:116701353 (rs76353203, **R19X,** nonsense, 2 carriers), 11:116701354 (rs138326449, **IVS2+1G->A**, splice, 3 carriers), 11:116701613 (rs140621530, **IVS3+1G->T**, splice, 5 carriers) and 11:116703578 (frameshift **CA->C**, 1 carrier). Two of these four variants (11:116701353 and 11:116701613) were validated through genotyping from the exome chip array and exhibited 100% concordance with the corresponding ARIC participant's exome sequence variant.

2.3. Outcomes

Stroke and CHD events were identified from continuous, comprehensive surveillance for all cardiovascular disease (CVD)-related hospitalizations and deaths in the 4 communities, and adjudicated on the basis of published criteria [24–28]. For this study CVD was defined as CHD plus hospitalized ischemic stroke. CHD was defined as the occurrence of definite or probable myocardial infarction, definite fatal CHD, or a coronary