Validation and Quantification of Genetic Determinants of Lipoprotein-a Levels and Predictive Value for Angiographic Coronary Artery Disease

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Lipoprotein(a) (Lp[a]) has gained attention as a heritable coronary artery disease (CAD) risk factor and therapeutic target. Two genetic variants in the LPA gene have been reported to influence Lp(a) levels and increase CAD risk. The aim of this study was to prospectively test these variants for their associations with Lp(a) and CAD risk. Participants (n = 1,400) in the Intermountain Heart Collaborative Study Registry who had Lp(a) cholesterol levels determined at coronary angiography were genotyped for rs3798220 and rs1045587 in LPA. Variants were detected by Taqman polymerase chain reaction. Chi-square and linear and logistic regression tests were used as appropriate among genotypes for Lp(a) and angiographic CAD. Age averaged 63 years; 65% were men; and severe CAD was present in 57%, mild CAD in 12%, and no CAD in 31%. Minor allele frequencies were 0.023 for rs3798220 and 0.090 for rs10455872. In multivariate modeling, only rs10455872 (odds ratio [OR] 2.33, 95% confidence interval [CI] 1.67 to 3.33, $p = 1.75 \times 10^{-9}$) and rs3798220 (OR 1.99, 95% CI 0.99 to 4.00, p = 0.065) contributed to the prediction of elevated Lp(a) cholesterol. Lp(a) cholesterol was weakly associated with CAD (OR 1.17, 95% CI 1.00 to 1.37, p = 0.055). Rs10455872 strongly predicted prevalent CAD (per allele OR 1.43, 95% CI 1.07 to 1.91, p = 0.0172); the effect size for the rare rs3798220 variant was similar (dominant OR 1.47, 95% CI 0.81 to 2.67, p = 0.20), but power was limited to demonstrate significance. The combined genotype explained only a small percentage (≤4%) of variability in Lp(a) cholesterol and prevalence of angiographic CAD. In conclusion, heritable contributions of LPA rs10455872 and rs3798220 to Lp(a) cholesterol levels and to angiographic CAD were prospectively assessed in this study. The percentage of intersubject variability in Lp(a) cholesterol and the percentage of prevalent CAD explained were small. **(Am J Cardiol 2013;112:799–804)**

Lipoprotein(a) (Lp[a]) is a complex circulating particle consisting of a large glycoprotein (apolipoprotein[a]) molecule, structurally resembling plasminogen, attached by a disulfide bond to an apolipoprotein B molecule.[1,2] Accumulating evidence suggests that Lp(a) is an independent risk factor for coronary artery disease (CAD), peripheral artery disease, and stroke[3–6] in whites and especially in blacks, who have a larger range of Lp(a),[7] leading some societies to endorse Lp(a) measurement to refine risk in selected populations.[8,9] Preclinical models suggest that Lp(a) is prothrombotic, proinflammatory, and proatherogenic,[10,11] and Lp(a) enters the arterial intima in humans.[12] Lp(a) concentrations are highly heritable, with a broad, bimodal concentration range. Genomewide association studies have discovered 2 common variants of the LPA gene, which encodes Lp(a) lipoprotein, that are associated with Lp(a) lipoprotein and increased CAD risk.[6,13,14] Despite these findings, the incremental value of Lp(a) measurement applied to cardiovascular risk refinement remains controversial.[15,16] Moreover, past experience with genetic studies suggests the need for multiple replications in distinct populations.[17]

Methods

To better define their clinical value for cardiovascular risk assessment, we tested 2 LPA variants (rs3798220 and rs10455872) for quantitative associations with Lp(a) levels and angiographic CAD in a prospectively enrolled and tracked patient cohort in the Intermountain Heart Collaborative Study Registry. On the basis of recent reports, we hypothesized (1) that Lp(a) cholesterol concentrations would be significantly associated with angiographic CAD and (2) that these 2 LPA single-nucleotide polymorphisms (SNPs) would be associated with Lp(a) concentrations and the risk for angiographic CAD. Our further intent was to prospectively quantify these associations in an independent and high-risk patient population with precise (angiographic) CAD phenotyping to determine the potential utility of genotyping for clinical CAD risk assessment and decision making.

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See page 803 for disclosure information.

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0002-9149/13/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.amjcard.2013.05.009

Participants (n = 1,400) in the Intermountain Heart Collaborative Study Registry who had Lp(a) cholesterol levels determined at the time of coronary angiography were entered into the study and were genotyped for rs3798220 and rs10455872 in LPA. All participants in the Intermountain Heart Collaborative Study Registry have given written informed consent for the use of plasma and deoxyribonucleic acid as well as demographic, medical, and angiographic characteristics in approved cardiovascular investigations. The Intermountain Heart Collaborative Study Registry and the present study were approved by the Intermountain Medical Center Institutional Review Board.

The Vertical Auto Profile test (Atherotech Diagnostic Laboratories, Birmingham, Alabama) was used to determine Lp(a) cholesterol. Unlike Lp(a) immunoassays, the Vertical Auto Profile test measures Lp(a) cholesterol directly and does not measure total particle mass. Normal values for Vertical Auto Profile Lp(a) cholesterol are <10 mg/dl. (As a result, Lp[a] cholesterol values are less than immunoassay numeric values.) An overview of previous study comparisons indicates that Lp(a) immunoassays and Lp(a) cholesterol correlate categorically (i.e., high vs normal or low) approximately 80% of the time (M. Cobble, MD, Atherotech, personal communication, August 27, 2012).

Genetic variants were detected by Taqman real-time polymerase chain reaction. Assays were obtained from Applied Biosystems (Foster City, California). The allele discrimination protocol was run on a Vii 7, real-time thermal cycler (Applied Biosystems). Five percent of samples were regenotyped for quality assurance. Successful genotyping was obtained for 94.6% of samples.

Coronary angiography was performed for clinical indications by experienced, board-certified cardiologists, who also visually assessed the presence and severity of CAD at the time of angiography and entered the results into a standardized electronic medical record form. Determination was blinded to Lp(a) cholesterol and genotype results. Three CAD categories were prospectively defined: none or minimal (<10% stenosis), mild to moderate (10% to 69% stenosis), and severe (≥70% stenosis in ≥1 major artery or a major [≥2 mm in diameter] branch).

Two methods were used to test for associations with Lp(a) cholesterol: one based on continuous Lp(a) cholesterol concentrations and another based on categorical Lp(a) cholesterol (normal, <10 mg/dl; abnormal, ≥10 mg/dl). Multivariate linear regression was used to test for associations between Lp(a) cholesterol (which was square-root transformed to achieve normality) and the LPA variants (additive model for rs10455872, dominant model for rs3798220) while adjusting for age, gender, history of hyperlipidemia (highly correlated with lipid-lowering medication or statin use), history of CAD, previous myocardial infarction, diabetes, smoking history, family history of early (age <60 years) CAD, and the natural logarithm of body mass index. Univariate chi-square tests and multivariate logistic regression were used as appropriate to test for associations between normal versus abnormal Lp(a) cholesterol and the LPA variants (additive model for rs10455872, dominant model for rs3798220). The multivariate model was again adjusted for age, gender, history of hyperlipidemia, history of CAD, previous myocardial infarction, diabetes, smoking history, family history of early CAD, and the natural log of body mass index. Chi-square tests were used to examine univariate associations between the variants and angiographic CAD, and logistic regression was applied to evaluate their associations in multivariate modeling, which adjusted for age, gender, hyperlipidemia, hypertension, diabetes, family history of premature CAD, smoking history, and the natural log of body mass index. In the latter models, CAD was defined as angiographic diameter stenosis ≥70% and non-CAD as the absence of visual angiographic stenosis (i.e., diameter stenosis <10%) and absent history of CAD, myocardial infarction, or coronary revascularization. (Those with mild to moderate CAD [diameter stenosis 10% to 69%] were excluded as an indeterminate phenotype for purposes of the present study.)

Table 1
Demographics of the study population (n = 1,400)

Variable	Value
Age (yrs)	62.8 ± 12.8
Men	915 (65.4%)
Race	
White	1,261 (90.1%)
Unknown race	70 (5.0%)
Hispanic	30 (2.1%)
Asian and Pacific Islander	17 (1.2%)
African American	11 (0.8%)
Other	11 (0.8%)
Body mass index (kg/m^2)	29.1 ± 6.6
Hyperlipidemia by history	771 (55.1%)
Hypertension by history	842 (60.1%)
Diabetes mellitus	314 (22.4%)
Smokers	244 (17.4%)
Previous CAD	583 (41.6%)
Previous myocardial infarction	87 (6.2%)
Previous heart failure	255 (18.2%)
Previous stroke	52 (3.7%)
Presentation with acute myocardial infarction	176 (12.6%)
Presentation with probable unstable angina pectoris	860 (61.4%)
Stable angina pectoris or non−chest pain presentation	364 (26.0%)
Systolic blood pressure (mm Hg)	145.9 ± 24.2
Diastolic blood pressure (mm Hg)	82.3 ± 13.1
Total cholesterol (mg/dl)	173.0 ± 41.8
High-density lipoprotein cholesterol (mg/dl)	41.2 ± 13.3
Low-density lipoprotein cholesterol (mg/dl)	102.5 ± 35.7
Triglycerides (mg/dl)	131 (94−183)
Lp(a) cholesterol (mg/dl)	5.57 ± 3.64
Extent of CAD at entry angiography	
None	434 (31.0%)
Mild to moderate*	165 (11.8%)
Severe	801 (57.2%)

Data are expressed as mean ± SD, number (percentage), or median (interquartile range).

* Mild to moderate CAD (i.e., 10% to 69% maximal coronary artery stenosis) was excluded from association analyses as an indeterminate phenotype.

Results

Demographics of the study population are listed in Table 1. Age averaged 63 years; 65% were men; and severe CAD was present in 57%, mild CAD in 12%, and no CAD