JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY
© 2014 BY THE AMERICAN COLLEGE OF CARDIOLOGY FOUNDATION
PUBLISHED BY ELSEVIER INC.

VOL. 64, NO. 9, 2014
ISSN 0735-1097/$36.00
http://dx.doi.org/10.1016/j.jacc.2014.03.061

ORIGINAL INVESTIGATIONS

Discrimination and Net Reclassification of Cardiovascular Risk With Lipoprotein(a)

Prospective 15-Year Outcomes in the Bruneck Study

Peter Willeit, MD, PhD,*† Stefan Kiechl, MD,* Florian Kronenberg, MD,‡ Joseph L. Witztum, MD, PhD,§ Peter Santer, MD,‖ Manuel Mayr, MD, PhD,¶ Qingbo Xu, MD, PhD,¶ Agnes Mayr, MD,‖ Johann Willeit, MD,* Sotirios Tsimikas, MD§

ABSTRACT

BACKGROUND Recent studies showed that lipoprotein(a) [Lp(a)] is a causal risk factor for cardiovascular disease (CVD). However, whether Lp(a) modifies clinical risk assessment was not established.

OBJECTIVES This study was conducted to determine whether Lp(a) improves CVD risk prediction.

METHODS In 1995, Lp(a) was measured in 826 men and women (age range, 45 to 84 years) from the general community. Incidence of CVD was recorded over 15 years of follow-up.

RESULTS In models adjusted for Framingham Risk Score (FRS) and Reynolds Risk Score (RRS) variables, the hazard ratio (HR) for incident CVD was 1.37 per 1-SD higher Lp(a) level (SD = 32 mg/dl) and 2.37 when comparing the top fifth quintile with other quintiles. The addition of Lp(a) to the RRS increased the C-index by 0.016. Of the 502 subjects who remained free of CVD, 82 were correctly reclassified to a lower risk category and 49 were reclassified to a higher risk category (predicted 15-year categories: <7.5%, 7.5% to <15%, 15% to <30%, ≥30%) ($p < 0.001$). Of the 148 subjects who developed CVD, 18 were correctly reclassified to a higher risk category and 17 were reclassified to a lower risk category. In subjects at intermediate risk (15% to <30%), the net reclassification improvement afforded by Lp(a) was 22.5% for noncases, 17.1% for cases, and 39.6% overall. Allele-specific Lp(a) levels did not add to the predictive ability of the FRS or RRS or to Lp(a).

CONCLUSIONS Elevated Lp(a) predicts 15-year CVD outcomes and improves CVD risk prediction. These findings suggest that Lp(a) levels may be used in risk assessment of subjects in the general community, particularly in intermediate-risk groups. (J Am Coll Cardiol 2014;64:851–60) © 2014 by the American College of Cardiology Foundation.

From the *Department of Neurology, Innsbruck Medical University, Innsbruck, Austria; †Department of Public Health and Primary Care, University of Cambridge, Cambridge, United Kingdom; ‡Department of Medical Genetics, Molecular and Clinical Pharmacology, Division of Genetic Epidemiology, Innsbruck Medical University, Innsbruck, Austria; §Department of Medicine, University of California San Diego, La Jolla, California; ‖Department of Laboratory Medicine, Bruneck Hospital, Bruneck, Italy; and the ¶King's British Heart Foundation Centre, King's College London, London, United Kingdom. This study was supported by National Institutes of Health (National Heart, Lung, and Blood Institute) grants R01-HL119828, R01-HL093767, P01-HL088093 and P01-HL055798 (Drs. Tsimikas and Witztum), by the Austrian Nationalbank (Project 9331). and by the Pustertaler Verein zur Prävention von Herz- und Hirngefaesserkrankungen, the Gesundheitsbezirk Bruneck, the mayor of Bruneck, and Assessorat für Gesundheit, Province of Bolzano, Italy. Drs. Tsimikas and Witztum are co-inventors and receive royalties from patents owned by the University of California San Diego on oxidation-specific antibodies and of biomarkers related to oxidized lipoproteins. Dr. Tsimikas is a consultant to ISIS, Genzyme, and Sanofi-Aventis; and has laboratory service agreements with Pfizer Inc. and Regeneron. Dr. Witztum is a consultant to ISIS, Regulus, and Intercept. All other authors have reported that they have no other relationships relevant to the contents of this paper to disclose.

Manuscript received November 7, 2013; revised manuscript received March 4, 2014, accepted March 11, 2014.

ABBREVIATIONS AND ACRONYMS

CAD = coronary artery disease

CI = confidence interval

CVD = cardiovascular disease

ERFC = Emerging Risk Factors Collaboration

FRS = Framingham Risk Score

HDL-C = high-density lipoprotein cholesterol

HR = hazard ratio

hs-CRP = high-sensitivity C-reactive protein

IDI = integrated discrimination improvement

KIV = kringle IV

LDL-C = low-density lipoprotein cholesterol

LMW = low-molecular-weight

Lp(a) = lipoprotein(a)

MI = myocardial infarction

NRI = net reclassification improvement

RRS = the Reynolds Risk Score

SNP = single nucleotide polymorphism

Lipoprotein(a) [Lp(a)] is composed of apolipoprotein B-100 and the covalently attached heterogeneous glycoprotein apolipoprotein(a). Lp(a) has no defined physiological function but is considered one of the strongest genetically determined risk factors for cardiovascular disease (CVD) (1). Circulating Lp(a) levels are mainly (~90%) genetically determined, primarily through variation in the *LPA* gene. Plasma Lp(a) levels can vary among individuals by more than 1,000-fold, mediated by copy number variation in the number of identical kringle IV type 2 (KIV-2) repeats and the presence of various single nucleotide polymorphisms (SNPs). Unlike in other lipoproteins, the influence of dietary and environmental factors on Lp(a) levels is small, and therefore Lp(a) is a quantitative genetic trait that influences atherogenicity from birth (2).

The mechanisms through which Lp(a) may be proatherogenic include the role of the low-density lipoprotein (LDL) moiety and the proinflammatory effects of the apo(a) component (1-3). Lp(a) also preferentially binds proinflammatory and proatherogenic oxidized phospholipids in comparison with other lipoproteins (4,5), induces macrophage apoptosis (6), and is integrally involved in initiating and potentially destabilizing atherosclerotic lesions (7). This finding is clinically substantiated by showing that the risk of Lp(a) is primarily driven by its content of oxidized phospholipids present in patients with high Lp(a) levels and small apo(a) isoforms (5,8,9).

SEE PAGE 861

The evidence for a causal role of Lp(a) in CVD is based on strong data from large epidemiological databases (10), mendelian randomization studies starting in the 1990s (11), and genome-wide association studies linking genetically determined Lp(a) levels to CVD events, such as by KIV-2 repeats or various SNPs influencing Lp(a) levels (12,13). Epidemiological studies suggest a log-linear relationship between circulating levels of Lp(a) and the risk of CVD that is independent of other lipid measures and conventional risk factors (14-18). Mendelian randomization studies demonstrate a causal, multivariable-adjusted, linear association between genetically determined Lp(a) levels and CVD risk (14,19,20). Finally, genome-wide association studies show that the *LPA* variants are strongly associated with reduced copy numbers of KIV-2 repeats, increased Lp(a) levels, and higher risk of coronary artery disease (CAD) (12,20).

These epidemiological and genetic studies suggest that measuring Lp(a) would enhance risk prediction and that Lp(a) should be a target of therapy (21). In that regard, it would be important to document that measuring Lp(a) levels allows clinicians to reclassify patients correctly into categories of lower or higher predicted risk using accepted risk algorithms. Therefore, the goal of this study was to assess whether Lp(a) allows reclassification of CVD risk by using a variety of methods. Because Lp(a) levels are genetically mediated, we assessed reclassification based on the Reynolds Risk Score (RRS), which additionally encompasses a family history of CVD and high-sensitivity C-reactive protein (hs-CRP).

METHODS

The Bruneck Study is a prospective population-based survey of the epidemiology and pathogenesis of atherosclerosis enrolling a sex- and age-stratified random sample of all inhabitants of Bruneck, Italy (125 women and 125 men, all in their fifth to eighth decade of age) (22). The current investigation focuses on blood samples from the 1995 examination and the follow-up period for clinical events between 1995 and 2010. In 1995, the study population consisted of 826 subjects, and plasma samples for assessment of Lp(a) were available in all participants. The ethics committees of Verona and Bolzano approved the study protocol, and all study subjects gave their written informed consent before entering the study. All risk factors were assessed by validated standard procedures.

STUDY METHODOLOGY AND LABORATORY METHODS. Study methodology and laboratory methods for factors listed in **Table 1** were previously described in detail (8,22-24). Determination of apolipoprotein(a) isoforms and measurement of allele-specific Lp(a) levels are available in the Online Appendix.

The primary composite CVD endpoint included vascular death (from ischemic stroke, myocardial infarction [MI], sudden cardiac death, or aortic aneurysm rupture), acute CAD (consisting of nonfatal MI, new-onset unstable angina defined as angina at rest, crescendo angina or new-onset severe angina, and acute coronary interventions), and ischemic stroke. MI was defined by the World Health Organization's criteria for definite disease status. Stroke was classified according to the criteria of the National Survey of Stroke. All other revascularization procedures (percutaneous intervention, bypass, and surgery) were carefully recorded. Ascertainment of

events or procedures did not rely on hospital discharge codes or the patient's self-report but rather on a careful review of medical records provided by the general practitioners and files of the Bruneck Hospital and the extensive clinical and laboratory examinations performed as part of the study protocols. Incident CVD events were ascertained from 1995 through 2010, and 100% follow-up was achieved.

Continuous variables were presented as mean ± SD or medians (interquartile range) and dichotomous variables as percentages. The CRP level was $\log_e$ transformed to obtain an approximately normal distribution of values. Differences in baseline levels of vascular risk attributes between subjects with and without subsequent CVD (1995 to 2010) were analyzed with the Student *t* test and the chi-square test. The association of baseline Lp(a) levels with CVD risk was quantified using Cox proportional hazard models, progressively adjusted for the Framingham Risk Score (FRS) variables (age, sex, diabetes, smoking, systolic blood pressure, total cholesterol, and high-density lipoprotein cholesterol [HDL-C]), and RRS (Framingham variables in addition to a parental history of premature MI and $\log_e$ CRP). Lp(a) was either entered as a continuous variable or categorized in quintiles. Proportional hazard assumptions were tested for Lp(a) by means of scaled Schoenfeld residuals and Cox models with time-dependent covariates and were satisfied. Differential associations in subgroups were analyzed by inclusion of appropriate interaction terms.

To assess the incremental predictive value for CVD risk afforded by Lp(a) measurement, this study used distinct approaches. First, the improvement in risk discrimination resulting from adding Lp(a) information to a model containing the RRS variables was quantified using Harrell's C-index (25). The C-index is suitable for time-to-event data and gives the probability that the model correctly predicts the order of failure of randomly selected pairs of individuals. A C-index of 1.0 indicates perfect prediction of the order of failure, whereas a C-index of 0.5 is achieved purely by chance. We used the individual RRS components rather than published formulas because the published formulas were for a 10-year period, a slightly different CVD endpoint, and nondiabetic subjects only. Using the individual variables rather than published scores is conservative because models with individual variables usually predict CVD better than do the scores, and it is more difficult for new variables to improve risk prediction. The reason this study did not exclude subjects with diabetes is that investigators have shown that the RRS works in diabetic patients as well (26); however, data are presented in a sensitivity analysis performed in persons without diabetes. The 95% confidence intervals (CIs) for C-indices and their changes were derived from jackknife standard errors (27). Comparison of the C-index for models including and not including information on Lp(a) was performed according to the method of DeLong (28) and with the Stata procedures somersd.

Second, we evaluated whether Lp(a) measurement helps correctly classify participants into categories of predicted CVD risk. Using the risk categories <7.5%, 7.5% to <15%, 15% to <30%, and ≥30%, the categorical net reclassification improvement (NRI) (25) was calculated for participants who experienced a CVD event within 15 years of follow-up (n = 148 in the study cohort) and for those who remained free of CVD throughout the entire survey period (n = 502). The authors also computed the prospective variant of the categorical NRI, which additionally includes

TABLE 1 Baseline Characteristics of Study Subjects (1995, N = 826) According to Incident CVD During Follow-Up (1995 to 2010)

	Primary Composite CVD Endpoint		
	No (n = 678)	Yes (n = 148)	p Value
Demographic parameters			
Age, yrs	66.6 ± 10.8	74.1 ± 10.5	<0.001
Female	352 (51.9)	60 (40.5)	0.014
Lipids, lipoproteins, and lipoprotein lipases			
Lp(a), mg/dl	23.3 ± 28.7	39.1 ± 43.8	<0.001
High Lp(a) (>45 mg/dl)*	114 (16.8)	51 (34.5)	<0.001
Apo(a) isoform, number of KIV repeats (major allele)†	25.3 ± 4.4	24.8 ± 4.7	0.208
Apo(a) isoform, ≤22 KIV repeats†	214 (31.7)	58 (39.5)	0.070
Total cholesterol, mg/dl‡	227.9 ± 41.1	237.5 ± 47.0	0.012
HDL cholesterol, mg/dl‡	59.0 ± 16.0	56.0 ± 16.9	0.049
LDL cholesterol, mg/dl‡	143.7 ± 36.6	152.9 ± 41.4	0.007
Lp-PLA_2 activity, μmol/min/l	765.3 ± 197.1	858.7 ± 215.8	<0.001
Vascular risk factors and inflammation			
Hypertension	451 (66.5)	113 (76.4)	0.025
Systolic blood pressure, mm Hg	146.9 ± 20.1	154.5 ± 22.3	<0.001
Diastolic blood pressure, mm Hg	86.9 ± 9.1	87.8 ± 9.8	0.265
Current smoking	137 (20.2)	24 (16.2)	0.303
Diabetes (ADA)	56 (8.3)	24 (16.2)	0.004
C-reactive protein, mg/l§	1.5 (0.8-3.0)	2.2 (1.1-4.8)	0.001
Body mass index, kg/m^2	25.6 ± 3.8	26.2 ± 4.1	0.081
Parental history of MI <60 yrs	20 (2.9)	4 (2.7)	0.871
Statin use			
Statin use at baseline	22 (3.2%)	6 (4.1%)	0.62
Statin use in 2010	124 (18.3%)	44 (29.7%)	0.002
Preexistent CVD			
CVD	47 (6.9)	41 (27.7)	<0.001

Values are mean ± SD or n (%). *High Lp(a) corresponds to the top fifth (i.e., >45 mg/dl). †Analysis was based on 822 participants with information on apo(a) isoform available with 147 incident CVD events. ‡To convert values for cholesterol to mmol/l, multiply by 0.02586. §Median and interquartile range is presented for high-sensitivity C-reactive protein.

ADA = American Diabetes Association; apo(a) = apolipoprotein(a); CVD = cardiovascular disease; HDL = high-density lipoprotein; KIV = kringle IV; LDL = low-density lipoprotein; Lp-PLA_2 = lipoprotein phospholipase A_2; Lp(a) = lipoprotein(a); MI = myocardial infarction.

participants who were censored before year 15 for causes unrelated to CVD (29). The standard errors for prospective NRI were estimated using 200 nonparametric bootstrap samples. Separate analyses focused on the continuous NRI, which does not depend on the arbitrary choice of categories, but deems any change in predicted risk in the correct direction as appropriate. Finally, we calculated the integrated discrimination improvement (IDI), which integrates the NRI over all possible cutoffs of predicted risk and mathematically corresponds to the difference in discrimination slopes of the 2 models in comparison (29,30). Statistical analyses were performed with SPSS 18.0 and Stata 12.0 MP software packages. All reported p values are 2-sided.

RESULTS

Table 1 displays the baseline characteristics of study subjects (n = 826) according to incident CVD during follow-up (1995 to 2010). Subjects developing the primary CVD endpoint were more likely to be older and male, have higher Lp(a) levels, and have a strong trend of <22 KIV repeats associated with CVD endpoints. They also had higher levels of lipid and inflammatory biomarkers and vascular risk factors. A parental history of MI was not different between the groups. The use of statins at baseline was similar among subjects with or without CVD. However, in the year 2010, more subjects with CVD were taking statins than were subjects without CVD (18.3% vs. 29.7%, p = 0.002). Baseline statin use was not related to apo(a) isoforms (3.3% in the ≤22 KIV group vs. 3.7% [p = 0.76] in the >22 KIV group). However, statin use during follow-up was higher in the ≤22 KIV group than in the >22 KIV group (26.1% vs 17.6%, p = 0.003, after adjustment for age and sex).

TABLE 2 Baseline Correlates of Lp(a) in the Bruneck Study (1995, N = 826)

	Mean ± SD or %	Age- and Sex-Adjusted Correlation Coefficient or Mean Difference in Lp(a) Levels Compared With Reference Category (95% CI)*
Continuous variables		
Age, yrs	68 ± 11	0.02 (−0.05 to 0.08)
Apo(a) isoform, KIV repeats	25 ± 5	−0.49 (−0.54 to −0.44)†
Total cholesterol, mg/dl	230 ± 42	0.20 (0.14 to 0.27)†
HDL cholesterol, mg/dl	58 ± 16	0.02 (−0.05 to 0.09)
Lp-PLA$_2$ activity, μmol/min/l	782 ± 204	0.09 (0.02 to 0.16)‡
Systolic blood pressure, mm Hg	148 ± 21	−0.01 (−0.07 to 0.06)
Log C-reactive protein, mg/l	1.8 ± 2.8	0.00 (−0.07 to 0.07)
Categorical variables		
Sex		
Male	50.1%	[Reference]
Female	49.9%	1.63 (−2.81 to 6.06)
Family history§		
No	97.1%	[Reference]
Yes	2.9%	3.20 (−10.04 to 16.45)
Diabetes mellitus		
No	90.3%	[Reference]
Yes	9.7%	−3.54 (−11.16 to 4.08)
Smoking status		
Never/ex-smoker	80.5%	[Reference]
Current	19.5%	2.75 (−3.00 to 8.51)

*Adjusted for age at baseline and sex. †p < 0.001. ‡p < 0.05. §Parental history of myocardial infarction before age 60 years.
CI = confidence interval; other abbreviations as in Table 1.

Correlates of baseline variables to Lp(a) showed no association with age, HDL-C, systolic blood pressure, or hs-CRP. Weak correlations were noted with total cholesterol and lipoprotein phospholipase A_2 activity (Table 2). A strong negative correlation was present between Lp(a) concentration and the number of KIV repeats of the smaller apo(a) isoforms (age- and sex-adjusted correlation coefficient, −0.49; 95% CI: −0.54 to −0.44; p = 5×10^{-59}) (Table 2).

Overall, 148 incident CVD events occurred (incidence rate, 15.0 per 1,000 person-years; 95% CI: 12.8 to 17.7). In a comparison of participants in the top fifth of Lp(a) levels to the remainder, the age- and sex-adjusted HR for CVD was 2.34 (1.67 to 3.29) (Table 3). The HR for CVD per 1-SD higher baseline level of Lp(a) was 1.38 (1.23 to 1.56) (SD = 32 mg/dl). Additional adjustment for smoking, systolic blood pressure, total cholesterol, HDL-C, diabetes, parental history of premature MI, and $\log_e$ hs-CRP did not materially change the effect estimates of this association (Table 3, Figure 1). Figure 2 displays a forest plot showing the multivariable adjusted risk of Lp(a) in various subgroups and demonstrates that Lp(a) predicts risk in all relevant subgroups with no evidence for differential associations (see Figure 2, p value for interaction >0.05 for all subgroup categories).

Lp(a) AND CARDIOVASCULAR RISK DISCRIMINATION AND RECLASSIFICATION. First, we evaluated the usefulness of Lp(a) in CVD risk discrimination. The C-index for CVD based on the FRS variables was 0.758 (0.722 to 0.794). Addition of apo(a) isoforms to this model did not change the C-index. Addition of Lp(a) to this model improved the C-index by 0.0164 (95% CI: 0.0019 to 0.0308; p = 0.027) (Figure 3). Addition of apo(a) isoforms to a model already containing FRS variables and Lp(a) did not further increase in the C-index, nor did replacement of "total" Lp(a) with allele-specific Lp(a) levels associated with low- versus high-molecular-weight (LMW vs. HMW) apo(a) isoforms. The C-index for CVD based on the RRS variables was 0.762 (0.726 top 0.798). Addition of information on Lp(a) to this model improved the C-index by 0.0155 (95% CI: 0.0014 to 0.0297;

TABLE 3 Association of Baseline Lp(a) Levels With the Risk of CVD Over 15 Years of Follow-Up (N = 826)*

Level of Adjustment	Hazard Ratio Comparing High Lp(a) Levels (Top Fifth, >45 mg/dl) to the Remainder		Hazard Ratio per 1-SD Higher Level of Lp(a)†	
	HR (95% CI)	p Value	HR (95% CI)	p Value
Progressive adjustment for Framingham Risk Score variables				
Adjustment for age and sex	2.34 (1.67–3.29)	<0.001	1.38 (1.23–1.56)	<0.001
Above + diabetes	2.34 (1.66–3.28)	<0.001	1.38 (1.22–1.56)	<0.001
Above + smoking	2.33 (1.66–3.28)	<0.001	1.38 (1.22–1.55)	<0.001
Above + systolic blood pressure	2.36 (1.68–3.32)	<0.001	1.38 (1.22–1.55)	<0.001
Above + total cholesterol	2.30 (1.63–3.24)	<0.001	1.36 (1.20–1.53)	<0.001
Above + HDL cholesterol (= Framingham Risk Score)	2.32 (1.65–3.27)	<0.001	1.37 (1.21–1.55)	<0.001
Further adjustment for all Reynolds Risk Score variables				
Above + parental history of premature MI	2.33 (1.66–3.29)	<0.001	1.36 (1.21–1.54)	<0.001
Above + $\log_e$ C-reactive protein (= Reynolds Risk Score)	2.37 (1.68–3.34)	<0.001	1.37 (1.21–1.55)	<0.001
Further adjustment for other variables				
Above + apo(a) isoform major allele (HMW vs. LMW)‡	2.61 (1.74–3.93)	<0.001	1.43 (1.23–1.67)	<0.001

*The primary composite cardiovascular endpoint subsumes ischemic stroke, acute coronary artery disease, and vascular death. †1 SD of Lp(a) corresponds to 32 mg/dll. ‡Analysis was based on 822 participants with 147 incident CVD events.

HMW = high molecular weight; HR = hazard ratio; LMW = low molecular weight; other abbreviations as in Tables 1 and 2.

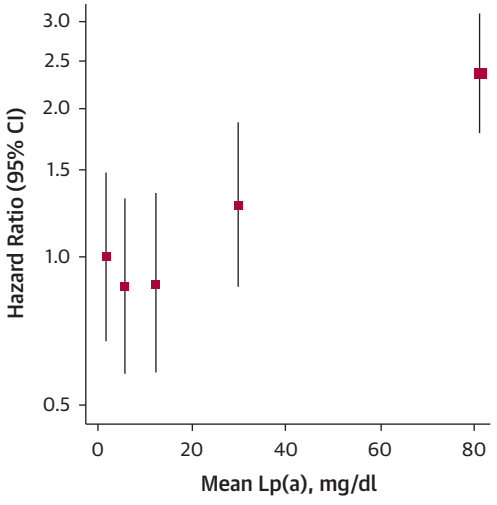


FIGURE 1 Shape of the Association Between Baseline Lp(a) Concentration and CVD Risk (826 Participants, 148 Incident CVD Events)

Hazard ratios across quintiles of lipoprotein(a) [Lp(a)] concentration were adjusted for the Reynolds Risk Score variables (i.e., age, sex, parental history of premature myocardial infarction, diabetes, smoking status, systolic blood pressure, total cholesterol, high-density lipoprotein cholesterol, and log C-reactive protein). The 95% confidence intervals (CIs) were estimated from the floated variances that reflect the amount of information underlying each group (including the reference group). Lp(a) quintiles were defined as: 0 to <3.2, 3.2 to <8.6, 8.6 to <17.7, 17.7 to <45, and ≥45 mg/dl. CVD = cardiovascular disease.

p = 0.031). Again, the corresponding C-index change for a combined use of information on Lp(a) and number of KIV repeats and allele-specific Lp(a) levels was unchanged (Figure 3). The change in C-index for CVD on use of Lp(a) was similar when the analysis was restricted to participants who were free of diabetes at baseline (ΔC-index, 0.0144; 95% CI: −0.0015 to 0.0304). Lp(a) improved the C-index from 0.756 to 0.779 if stroke was used as the endpoint (ΔC-index, 0.0232; 95% CI: 0.0004 to 0.0459; p = 0.046; 65 incident stroke events). C-index (0.0158 [95% CI: 0.0015 to 0.0301]) and retrospective NRI (7.73 [95% CI: −1.38 to 16.83]) for "corrected" allele-specific Lp(a) levels were nearly identical.

Second, we evaluated the usefulness of Lp(a) in CVD risk reclassification. Table 4 shows the classification of individuals based on RRS variables alone and in combination with Lp(a) into categories of predicted 15-year risk (<7.5%; 7.5% to <15%; 15% to <30%; ≥30%). Of the 502 subjects who remained free of CVD, 82 were correctly reclassified to a lower-risk category and 49 were reclassified to a higher-risk category. Of the 148 subjects who developed CVD, 18 were correctly reclassified to a higher-risk category and 17 were reclassified to a lower-risk category.

Third, we calculated the NRI and IDI for CVD (Table 5). According to the foregoing data, the categorical retrospective NRI was 0.7% in cases (p = 0.866), 6.6% in noncases (p = 0.004), and 7.3% overall (p = 0.115). In comparison, the categorical prospective NRI was 5.8% (p = 0.217), the continuous NRI was 20.7% (p = 0.046), and the IDI was 0.027

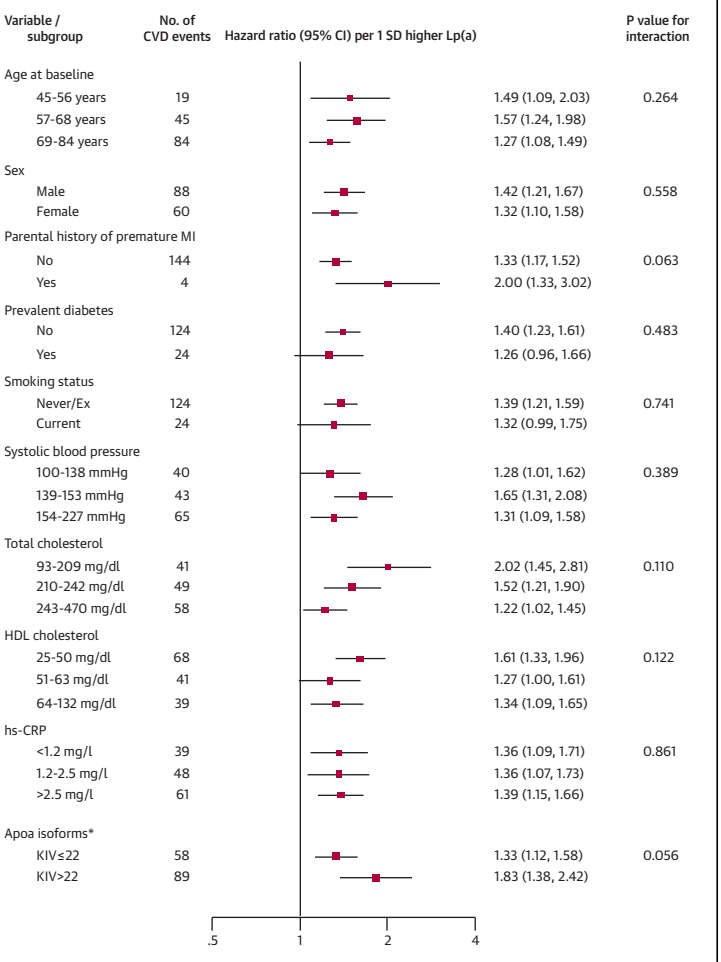


FIGURE 2 Association of Lp(a) With Cardiovascular Risk in Clinically Relevant Subgroups

Hazard ratios were adjusted for age, sex, parental history of premature myocardial infarction (MI), diabetes, smoking status, systolic blood pressure, total cholesterol, high-density lipoprotein (HDL) cholesterol, and log C-reactive protein (high-sensitivity CRP [hs-CRP]). *Analysis was based on 822 participants with 147 incident cardiovascular disease (CVD) events. KIV, number of kringle IV repeats. Apo(a) = apolipoprotein(a); other abbreviations as in Figure 1.

($p = 0.006$). Results were comparable when the analysis was restricted to participants without diabetes at baseline (Table 5). The classification gain of a targeted assessment of Lp(a) in the intermediate risk group (15% to ≤30% predicted 15-year risk of CVD) was as follows: 22.5% for noncases (95% CI: 10.6 to 34.4; $p < 0.001$), 17.1% for cases (95% CI: −1.4 to 35.6; $p = 0.071$), and 39.6% overall (95% CI: 17.6 to 61.6; $p < 0.001$).

Because smaller apo(a) isoforms expressed in low quantity could add significant weight in such analyses, we have performed a supplementary analysis that used the isoform for categorization in LMW and HMW apo(a) phenotypes that was most strongly expressed in the immunoblot. Analysis using this variable showed a slightly stronger association between LMW apo(a) and CVD (35.8% vs. 26.8% in subjects with and without incident CVD, $p = 0.028$) but virtually identical results regarding risk discrimination and reclassification (data not shown).

DISCUSSION

This study demonstrates that adding Lp(a) to the FRS and RRS improves discrimination and reclassification of CVD risk over prospective 15-year follow-up in an unselected population. Because this cohort reflects patients who are seen in a primary care setting, the findings suggest that Lp(a) levels may be used in risk assessment in patients in the general community, particularly those at intermediate risk of CVD (**Central Illustration**).

Debate on the role of clinical testing of Lp(a) levels is considerable, and until recently, no guidelines had been published to guide clinical decision making. In 2010, the European Atherosclerosis Society recommended Lp(a) measurement once in patients with premature CVD, as well as those in the following categories: intermediate or high CVD risk, familial hypercholesterolemia, family history of premature CVD and/or elevated Lp(a), recurrent CVD despite statin treatment, ≥3% 10-year risk of fatal CVD (according to European guidelines), and/or ≥10% 10-year risk of fatal and nonfatal coronary heart disease (according to U.S. guidelines) (15). They further recommended that a secondary priority after low-density lipoprotein cholesterol (LDL-C) lowering is a desirable Lp(a) level of <50 mg/dl (<80th percentile) and, based on meta-analyses but no prospective Lp(a)-specific lowering studies, recommended treatment with niacin to reduce Lp(a) below this level. The U.S. National Lipid Association provided similar recommendations on testing, but no recommendation on desirable levels or therapy (31). These recommendations remain controversial pending a randomized trial that enrolls patients with high Lp(a) levels and specifically and potently lowers Lp(a) and reduces clinical outcomes. However, they add an important voice to the debate and will stimulate needed clinical research on reducing the CVD risk of Lp(a).

This study's findings support the notion that Lp(a) is useful in clinical risk prediction in the primary care setting. This was particularly true in subjects at intermediate CVD risk, whose categorical NRI afforded by Lp(a) was 39.6% overall (22.5% for noncases and 17.1% for cases). Additional measures of NRI and IDI were consistent with the foregoing.

Measures of reclassification that do not rely on the choice of cutoff values (continuous NRI and IDI) indicated a significant gain in classification accuracy when Lp(a) level was added to the RRS (Table 5). The continuous NRI deems any change in predicted risk in the correct direction as appropriate and thus avoids arbitrary cutoffs, whereas the IDI integrates the net reclassification over all possible cutoffs of predicted risk. Categorical NRIs using predefined categories were significant for noncases only (Tables 4 and 5). Categorical NRIs are commonly reported for their ease of interpretation but are sensitive to the arbitrary choice of cutoff values. Overall, the study suggests a worthwhile gain in CVD risk discrimination and classification based on a single assessment of Lp(a), which varies minimally through a person's lifetime around a preset genetically determined level. This seems to be an adequate investment in resources, although a formal cost analysis remains to be performed.

Prior studies evaluating the role of Lp(a) in CVD risk discrimination and reclassification in accepted prediction models such as RRS have not been performed, but 2 recent studies have addressed similar questions (16,17). In an investigation by the Emerging Risk Factors Collaboration (ERFC), based on data from 24 prospective studies and 133,502 participants (12,639 incident CVD events), Lp(a) was shown to reclassify 4.1% of subjects when it was added to total cholesterol and HDL-C (16). The C-index for Lp(a) increased from 0.7229 to 0.7245. The C-index also increased significantly in the intermediate-risk (by 0.0089) and high-risk FRS categories (by 0.0026). These values are more modest compared with the current study in which the C-index increased from 0.7580 to 0.7744, and we note that the increase in the C-index for stroke was even more impressive, increasing from 0.756 to 0.779. Limitations of the ERFC analysis include the lack of evaluation of clinical risk scores and concomitant data on Lp(a) and apo(a) isoforms, which made an evaluation of the benefits of a combined use of information on these markers impossible. Kamstrup et al. (17) studied 8,720 participants (1,683 incident CVD events) and evaluated the role of extreme Lp(a) levels (80th and 95th percentiles) in CVD risk. The HRs were similar to those in the current study: Lp(a) at 80th percentile (≥47 mg/dl) had a HR of 1.5 for CVD and at the 95th percentile (≥117 mg/dl) has a HR of 1.7. When adding Lp(a) to conventional risk factors, the NRI was 3% and 6%, and the IDI 0.015 and 0.022 for the 80th and 95th percentile, respectively, similar to the current study. However, unlike the current analysis, when adding Lp(a) over the entire range of concentrations,

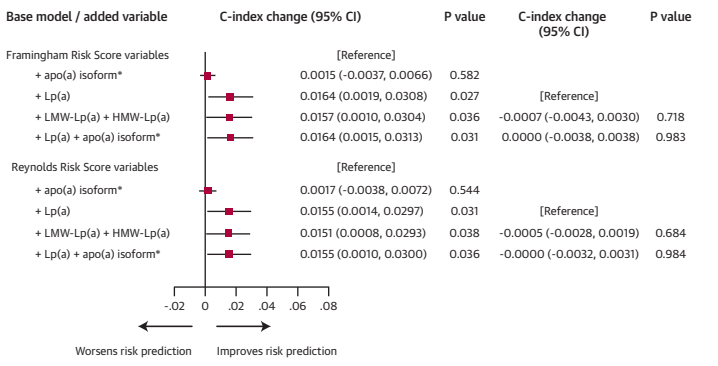


FIGURE 3 Improvement of Discrimination of CVD Risk With the Use of Single and Combined Information on Lp(a) Level and apo(a) Isoform

This analysis focuses on the 822 subjects with both lipoprotein(a) [Lp(a)] level and apolipoprotein(a) [apo(a)] isoform available. *Apo(a) isoform was included as a dichotomous variable of low molecular weight (LMW ≤22 kringle IV [KIV] repeats) versus high molecular weight (HMW >22 KIV repeats), and this dichotomization refers to the smaller of the 2 apo(a) alleles in case both alleles are expressed. Abbreviations as in Figures 1 and 2.

NRI did not improve, but IDI did improve slightly. *LPA* genotypes were measured as the sum of KIV-2 repeats of the two alleles (which underestimates the effect of the dominant isoform) or by *LPA* SNPs. *LPA* genotypes also improved NRI and IDI, but the

TABLE 4 Reclassification of Study Participants Into Groups With Low, Medium, and High 15-Year Risk of CVD by Addition of Lp(a) to a Model Containing the Reynolds Risk Score Variables*

Participants Who Experienced a CVD Event (n = 148)

Model With Reynolds Score Variables	Model With Reynolds Risk Score Variables Plus Lp(a)				
	Total	<7.5% Risk	7.5% to <15% Risk	15% to <30% Risk	≥30% Risk
<7.5% risk	9	7 (77.8)	2 (22.2)†	0 (0.0)†	0 (0.0)†
7.5% to <15% risk	18	1 (5.6)‡	12 (66.7)	5 (27.8)†	0 (0.0)†
15% to <30% risk	41	0 (0.0)‡	4 (9.8)‡	26 (63.4)	11 (26.8)†
≥30% risk	80	0 (0.0)‡	0 (0.0)‡	12 (15.0)‡	68 (85.0)
Total	148	8	18	43	79

Participants Who Remained Event-Free (n = 502)

	Model With Reynolds Risk Score Variables Plus Lp(a)				
	Total	<7.5% Risk	7.5% to <15% Risk	15% to <30% Risk	≥30% Risk
<7.5% risk	180	166 (92.2)	13 (7.2)§	1 (0.6)§	0 (0)§
7.5% to ≥15% risk	145	27 (18.6)‖	96 (66.2)	22 (15.2)§	0 (0)§
15% to <30% risk	120	0 (0)‖	40 (33.3)‖	67 (55.7)	13 (10.8)§
≥30% risk	57	0 (0)‖	0 (0)‖	15 (26.3)‖	42 (73.7)
Total	502	193	149	105	55

Values are n (row %). *This reclassification table compares a model based on the Reynolds Risk Score only with a model considering the Reynolds Risk Score plus Lp(a) level. NRI denotes the classic retrospective categorical net reclassification improvement with calculations based on 148 subjects with and 502 subjects without events and a complete follow-up over the 15-year period. †Moved to higher risk, n = 18; ‡Moved to lower risk, n = 17; NRI 0.68% (−7.16, 8.51). §Moved to higher risk, n = 49; ‖Moved to lower risk, n = 82; NRI 6.57% (2.11, 11.04).

NRI = net reclassification improvement; other abbreviations as in Table 1.

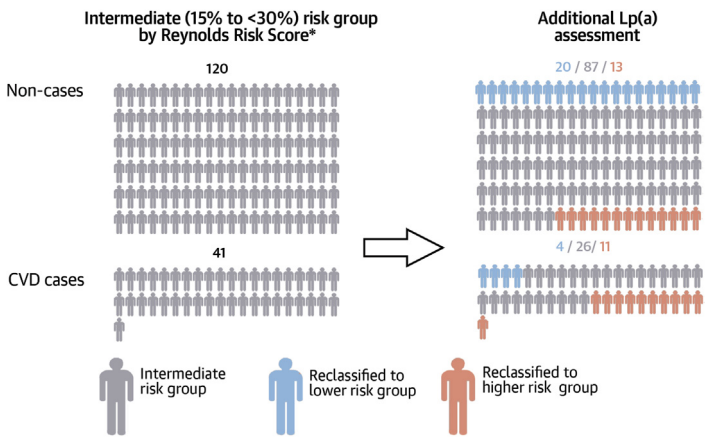
TABLE 5 Improvement in 15-Year CVD Risk Prediction by Use of Lp(a) on Top of a Model Containing the Reynolds Risk Score Variables*

	Change of Risk Prediction Metrics (95% CI) on Addition of Lp(a)	
	Overall Cohort	Exclusion of Participants With Diabetes
No. of participants	826	746
No. of incident CVD events	148	124
Categorical retrospective NRI	7.3 (−1.8 to 16.3)	7.9 (−1.8 to 17.7)
Noncases	6.6 (2.3 to 11.0)	5.5 (0.8 to 10.2)
Cases	0.7 (−7.2 to 8.5)	2.4 (−6.1 to 10.9)
Categorical prospective NRI	5.8 (−3.4 to 14.7)	6.8 (−3.9 to 17.5)
Noncases	7.5 (3.9 to 11.1)	6.7 (3.0 to 10.4)
Cases	−1.8 (−10.2 to 6.7)	0.1 (−9.7 to 9.9)
Continuous NRI	20.7 (0.3 to 41.1)	22.7 (2.4 to 43.0)
IDI (95% CI)	0.027 (0.008 to 0.047)	0.034 (0.013 to 0.056)
Categorical retrospective NRI Intermediate risk group	39.6 (17.6 to 61.6)	32.8 (9.3 to 56.2)
Noncases	22.5 (10.6 to 34.4)	18.9 (6.1 to 31.7)
Cases	17.1 (−1.4 to 35.6)	13.9 (−5.7 to 33.5)

*Age, sex, diabetes, systolic blood pressure, total cholesterol, high-density lipoprotein cholesterol, parental history of myocardial infarction at <60 years of age, and $\log_e$-transformed C-reactive protein level.

IDI = integrated discrimination improvement; other abbreviations as in Tables 1, 2, and 4.

combination of Lp(a) levels and *LPA* genotypes did not enhance NRI or IDI for coronary heart disease and slightly enhanced it for MI. Although differences exist among the current study and the 2 foregoing studies, they are all remarkably consistent in enhancing risk prediction by adding Lp(a) to conventional risk factors.



CENTRAL ILLUSTRATION Reclassification of Individuals Predicted to be at Intermediate 15-Year CVD Risk by Additional Assessment of Lp(a)

Predicted risk groups were defined as: lower-risk group <15%; intermediate-risk group 15 to <30%; and higher-risk group ≥30%. *The Reynolds Risk Score contains information on age, sex, diabetes, smoking, systolic blood pressure, total cholesterol, high-density lipoprotein cholesterol, parental history of premature myocardial infarction, and $\log_e$ C-reactive protein. CVD = cardiovascular disease; Lp(a) = lipoprotein(a).

In the current study, neither apo(a) isoforms nor allele-specific Lp(a) levels (32) added to the predictive ability of FRS or RRS, nor to Lp(a), unlike earlier studies (11,33). In a prior study from Bruneck that evaluated 5-year progression of carotid atherosclerosis assessed by high-resolution duplex ultrasound, the presence of LMW apo(a) phenotypes emerged as one of the strongest risk predictors of advanced stenotic atherosclerosis, especially when it was associated with high Lp(a) plasma concentrations (24). In the current study, we instead evaluated the capability of apo(a) phenotypes to predict CVD events in clinical practice. The discrepancy in findings between anatomic disease and CVD events, however, may have been confounded over the next 10-year follow-up by the issue that subjects with LMW isoforms were more likely to be treated with statins. Higher use of statins in patients with LMW isoforms may have abrogated the CVD effect of LMW apo(a) isoforms, as suggested by a similar attenuation of Lp(a) effects in a recent meta-analysis in 18,979 patients with established CAD who were receiving statin therapy (34). A meta-analysis by Erqou et al. (19) in 40 studies and 58,000 subjects demonstrated a relative risk of 2.08 for coronary heart disease for individuals with ≤22 KIV-2 repeats versus >22 repeats. However, Lp(a) levels were not generally available for adjustment, and therefore the true effect of apo(a) isoforms added to Lp(a) levels could not be determined. In the Framingham Offspring Study, adding apo(a) isoforms to Lp(a) levels did not add predictive value (35). Finally, various SNPs have been shown to affect Lp(a) levels, but they do not seem to predict CVD risk independently when Lp(a) is added to the models (12,17). Overall, these studies suggest that Lp(a) is the main driver of CVD risk and that the additive effect of apo(a) isoforms to the predictive ability of Lp(a) is likely modest. However, further studies are needed in a variety of clinical groups with genetically different isoform distributions, particularly those with small isoforms, to determine point estimates more accurately.

In view of accumulating data from epidemiological, genetic association, and mendelian randomization studies, Lp(a) has become a target of therapy (21). The antisense inhibitor mipomersen, approved to lower LDL-C levels in patients with homozygous familial hypercholesterolemia, lowers Lp(a) levels 21% to 39% (36). Cholesterol ester transferase protein (CETP) inhibitors and proprotein convertase subtilisin/kexin type 9 (PCSK9) monoclonal antibodies currently in trials also lower Lp(a) up to 40% (21,37). Finally, specific antisense inhibitors to apo(a) have been tested in both animal models (38) and in phase I

trials and lower Lp(a) up to 95% (39). Such drugs may allow testing of the clinical hypothesis that lowering Lp(a) levels in patients with elevated Lp(a) levels may result in clinical benefit.

STUDY STRENGTHS. The presented data were of high quality, including the comprehensive assessment of measurements at baseline in all individuals and 100% follow-up for clinical endpoints over a period of 15 years. More detailed analyses were performed with regard to the targeted use of Lp(a) in the intermediate-risk group and comparisons for cases and controls. All participants of the study were Caucasian. Lp(a) SNPs were not available, but previous studies showed that the association between SNPs and CVD disappears after adjustment for Lp(a) (12,17). Thus, no reason exists to assume that Lp(a) SNPs improve risk prediction beyond the information provided by Lp(a) level and apo(a) isoforms. This is especially true considering that less than half of the LMW apo(a) isoforms are tagged by the currently used SNPs (40).

STUDY LIMITATIONS. Risk reclassification metrics (e.g., NRI) have inherent limitations, such as dependence on the choice of cutoff and time frame for predicted risks and on the age distribution of the study population. NRIs were presented following published recommendations (41), and they yielded findings consistent with other risk prediction metrics.

CONCLUSIONS

Elevated Lp(a) predicts 15-year CVD outcomes and improves CVD risk prediction. These findings suggest that Lp(a) levels may be used in risk assessment of subjects in the general community, particularly in intermediate-risk groups.

REPRINT REQUESTS AND CORRESPONDENCE: Dr. Sotirios Tsimikas, Vascular Medicine Program, University of California San Diego, 9500 Gilman Drive, BSB 1080, La Jolla, California 92093-0682. E-mail: stsimikas@ucsd.edu OR Dr. Stefan Kiechl, Department of Neurology, Medical University Innsbruck, Innsbruck, Austria. E-mail: Stefan.Kiechl@i-med.ac.at.

PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Although Lp(a) has been identified as a causal risk factor for CVD, its predictive value has not been fully established.

TRANSLATIONAL OUTLOOK: The clinical utility of basing clinical management decisions on Lp(a) measurements for primary prevention of cardiovascular events awaits evaluation in prospective clinical trials.

REFERENCES

1. Kronenberg F, Utermann G. Lipoprotein(a): resurrected by genetics. J Intern Med 2013;273:6–30.

2. Tsimikas S, Hall JH. Lipoprotein(a) as a potential causal genetic risk factor of cardiovascular disease: a rationale for increased efforts to understand its pathophysiology and develop targeted therapies. J Am Coll Cardiol 2012;60:716–21.

3. Spence JD, Koschinsky ML. Mechanisms of lipoprotein(a) pathogenicity. Arterioscler Thromb Vasc Biol 2012;32:1550–1.

4. Bergmark C, Dewan A, Orsoni A, et al. A novel function of lipoprotein [a] as a preferential carrier of oxidized phospholipids in human plasma. J Lipid Res 2008;49:2230–9.

5. Leibundgut G, Scipione C, Yin H, et al. Determinants of binding of oxidized phospholipids on apolipoprotein (a) and lipoprotein (a). J Lipid Res 2013;54:2815–30.

6. Seimon TA, Nadolski MJ, Liao X, et al. Atherogenic lipids and lipoproteins trigger CD36-TLR2-dependent apoptosis in macrophages undergoing endoplasmic reticulum stress. Cell Metab 2010;12:467–82.

7. Lee S, Birukov KG, Romanoski CE, Springstead JR, Lusis AJ, Berliner JA. Role of phospholipid oxidation products in atherosclerosis. Circ Res 2012;111:778–99.

8. Tsimikas S, Willeit P, Willeit J, et al. Oxidation-specific biomarkers, prospective 15-year cardiovascular and stroke outcomes, and net reclassification of cardiovascular events. J Am Coll Cardiol 2012;60:2218–29.

9. Tsimikas S, Duff GW, Berger PB, et al. Pro-inflammatory interleukin-1 genotypes potentiate the risk of coronary artery disease and cardiovascular events mediated by oxidized phospholipids and lipoprotein (a). J Am Coll Cardiol 2014;63:1724–34.

10. Erqou S, Kaptoge S, Perry PL, et al. Lipoprotein(a) concentration and the risk of coronary heart disease, stroke, and nonvascular mortality. JAMA 2009;302:412–23.

11. Sandholzer C, Saha N, Kark JD, et al. Apo(a) isoforms predict risk for coronary heart disease: a study in six populations. Arterioscler Thromb 1992;12:1214–26.

12. Clarke R, Peden JF, Hopewell JC, et al. Genetic variants associated with Lp(a) lipoprotein level and coronary disease. N Engl J Med 2009;361:2518–28.

13. Lanktree MB, Anand SS, Yusuf S, Hegele RA. Comprehensive analysis of genomic variation in the LPA locus and its relationship to plasma lipoprotein(a) in South Asians, Chinese, and European Caucasians. Circ Cardiovasc Genet 2010;3:39–46.

14. Kamstrup PR, Tybjaerg-Hansen A, Steffensen R, Nordestgaard BG. Genetically elevated lipoprotein(a) and increased risk of myocardial infarction. JAMA 2009;301:2331–9.

15. Nordestgaard BG, Chapman MJ, Ray K, et al. Lipoprotein(a) as a cardiovascular risk factor: current status. Eur Heart J 2010;31:2844–53.

16. Di Angelantonio E, Gao P, Pennells L, et al. Lipid-related markers and cardiovascular disease prediction. JAMA 2012;307:2499–506.

17. Kamstrup PR, Tybjaerg-Hansen A, Nordestgaard BG. Extreme lipoprotein(a) levels and improved cardiovascular risk prediction. J Am Coll Cardiol 2013;61:1146–56.

18. Bertoia ML, Pai JK, Lee JH, et al. Oxidation-specific biomarkers and risk of peripheral artery disease. J Am Coll Cardiol 2013;61:2169–79.

19. Erqou S, Thompson A, Di Angelantonio E, et al. Apolipoprotein(a) isoforms and the risk of vascular disease: systematic review of 40 studies involving 58,000 participants. J Am Coll Cardiol 2010;55:2160–7.

20. Helgadottir A, Gretarsdottir S, Thorleifsson G, et al. Apolipoprotein(a) genetic sequence variants

associated with systemic atherosclerosis and coronary atherosclerotic burden but not with venous thromboembolism. J Am Coll Cardiol 2012;60:722–9.

21. Kolski B, Tsimikas S. Emerging therapeutic agents to lower lipoprotein (a) levels. Curr Opin Lipidol 2012;23:560–8.

22. Kiechl S, Willeit J. The natural course of atherosclerosis. Part I: incidence and progression. Arterioscler Thromb Vasc Biol 1999;19:1484–90.

23. Tsimikas S, Kiechl S, Willeit J, et al. Oxidized phospholipids predict the presence and progression of carotid and femoral atherosclerosis and symptomatic cardiovascular disease: five-year prospective results from the Bruneck study. J Am Coll Cardiol 2006;47:2219–28.

24. Kronenberg F, Kronenberg MF, Kiechl S, et al. Role of lipoprotein(a) and apolipoprotein(a) phenotype in atherogenesis: prospective results from the Bruneck study. Circulation 1999;100:1154–60.

25. Pencina MJ, D'Agostino RB Sr, D'Agostino RB Jr, Vasan RS. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. Stat Med 2008;27:157–72; discussion 207–12.

26. Cook NR, Paynter NP, Eaton CB, et al. Comparison of the Framingham and Reynolds risk scores for global cardiovascular risk prediction in the multiethnic women's health initiative. Circulation 2012;125:1748–56.

27. Newson R. Efficient calculation of jackknife confidence intervals for rank statistics. J Stat Softw 2006;15:1–10.

28. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics 1988;44:837–45.

29. Pencina MJ, D'Agostino RB Sr, Steyerberg EW. Extensions of net reclassification improvement calculations to measure usefulness of new biomarkers. Stat Med 2011;30:11–21.

30. Steyerberg EW, Vickers AJ, Cook NR, et al. Assessing the performance of prediction models: a framework for traditional and novel measures. Epidemiology 2010;21:128–38.

31. Davidson MH, Ballantyne CM, Jacobson TA, et al. Clinical utility of inflammatory markers and advanced lipoprotein testing: advice from an expert panel of lipid specialists. J Clin Lipidol 2011;5:338–67.

32. Enkhmaa B, Anuurad E, Zhang W, et al. HIV disease activity as a modulator of lipoprotein(a) and allele-specific apolipoprotein(a) levels. Arterioscler Thromb Vasc Biol 2013;33:387–92.

33. Rifai N, Ma J, Sacks FM, et al. Apolipoprotein(a) size and lipoprotein(a) concentration and future risk of angina pectoris with evidence of severe coronary atherosclerosis in men: the Physicians' Health Study. Clin Chem 2004;50:1364–71.

34. O'Donoghue ML, Morrow DA, Tsimikas S, et al. Lipoprotein(a) for risk assessment in patients with established coronary artery disease. J Am Coll Cardiol 2014;63:520–7.

35. Lamon-Fava S, Marcovina SM, Albers JJ, et al. Lipoprotein(a) levels, apo(a) isoform size, and coronary heart disease risk in the Framingham Offspring Study. J Lipid Res 2011;52:1181–7.

36. Tsimikas S, Witztum J, Catapano A. Effect of mipomersen on lipoprotein(a) in patients with hypercholesterolemia across four phase III studies (abstr). J Am Coll Cardiol 2012;59 Suppl:A366.

37. McKenney JM, Koren MJ, Kereiakes DJ, Hanotin C, Ferrand AC, Stein EA. Safety and efficacy of a monoclonal antibody to proprotein convertase subtilisin/kexin type 9 serine protease, SAR236553/REGN727, in patients with primary hypercholesterolemia receiving ongoing stable atorvastatin therapy. J Am Coll Cardiol 2012;59:2344–53.

38. Merki E, Graham M, Taleb A, et al. Antisense oligonucleotide lowers plasma levels of apolipoprotein (a) and lipoprotein (a) in transgenic mice. J Am Coll Cardiol 2011;57:1611–21.

39. Viney NJ, Graham M, Crooke R, Hughes S, Singleton W. Evaluation of Isis apo(a)Rx, an antisense inhibitor to apolipoprotein(a), in healthy volunteers. Circulation 2013;128:A14196.

40. Laminca C, Kronenberg F. The mysterious lipoprotein(a) is still good for a surprise. Lancet Diabetes Endocrinol 2013;1:170–2.

41. Kerr KF, Wang Z, Janes H, McClelland RL, Psaty BM, Pepe MS. Net reclassification indices for evaluating risk prediction instruments: a critical review. Epidemiology 2014;25:114–21.

KEY WORDS atherosclerosis, autoantibodies, lipoproteins, oxidation, oxidation-specific epitopes, oxidized phospholipids

APPENDIX For supplemental material, please see the online version of this article.