

Population and assay thresholds for the predictive value of lipoprotein (a) for coronary artery disease: the EPIC-Norfolk Prospective Population Study¹

Rutger Verbeek,* S. Matthijs Boekholdt,[†] Robert M. Stoekenbroek,* G. Kees Hovingh,* Joseph L. Witztum,[§] Nicholas J. Wareham,** Manjinder S. Sandhu,^{††,§§} Kay-Tee Khaw,^{††} and Sotirios Tsimikas^{2,***}

Department of Vascular Medicine* and Department of Cardiology,[†] Academic Medical Center, Amsterdam, The Netherlands; Division of Endocrinology, Department of Medicine,[§] University of California, San Diego, La Jolla, CA; Medical Research Council Epidemiology Unit,** Cambridge, United Kingdom; Department of Public Health and Primary Care,^{††} University of Cambridge, Cambridge, United Kingdom; Genetic Epidemiology Group,^{§§} Wellcome Trust Sanger Institute, Hinxton, United Kingdom; and Vascular Medicine Program, Sulpizio Cardiovascular Center,^{***} University of California, San Diego, La Jolla, CA

Abstract Variable agreement exists between different lipoprotein (a) [Lp(a)] measurement methods, but their clinical relevance remains unclear. The predictive value of Lp(a) measured by two different assays [Randox and University of California, San Diego (UCSD)] was determined in 623 coronary artery disease (CAD) cases and 948 controls in a case-control study within the EPIC-Norfolk Prospective Population Study. Participants were divided into sex-specific quintiles, and by Lp(a) <50 versus \geq 50 mg/dl, which represents the 80th percentile in northern European subjects. Randox and UCSD Lp(a) levels were strongly correlated; Spearman's correlation coefficients for men, women, and sexes combined were 0.905, 0.915, and 0.909, respectively ($P < 0.001$ for each). The >80th percentile cutoff values, however, were 36 mg/dl and 24 mg/dl for the Randox and UCSD assays, respectively. Despite this, Lp(a) levels were significantly associated with CAD risk, with odds ratios of 2.18 (1.58–3.01) and 2.35 (1.70–3.26) for people in the top versus bottom Lp(a) quintile for the Randox and UCSD assays, respectively. This study demonstrates that CAD risk is present at lower Lp(a) levels than the currently suggested optimal Lp(a) level of <50 mg/dl. **Appropriate thresholds may need to be population and assay specific until Lp(a) assays are standardized**

and Lp(a) thresholds are evaluated broadly across all populations at risk for CVD and aortic stenosis.—Verbeek, R., S. M. Boekholdt, R. M. Stoekenbroek, G. K. Hovingh, J. L. Witztum, N. J. Wareham, M. S. Sandhu, K-T. Khaw, and S. Tsimikas. **Population and assay thresholds for the predictive value of lipoprotein (a) for coronary artery disease: the EPIC-Norfolk Prospective Population Study.** *J. Lipid Res.* 2016. 57: 697–705.

Supplementary key words atherosclerosis • aortic stenosis • risk factor

Elevated levels of lipoprotein (a) [Lp(a)] are a genetic, independent risk factor for the development of CVD (1). The European Atherosclerosis Society (EAS) Consensus Panel has recommended that Lp(a) levels should be measured in patients with an intermediate or high CVD risk and that desirable Lp(a) levels are below the 80th percentile of the population distribution, which roughly corresponded to 50 mg/dl in a Copenhagen population of 6,000 subjects (2). Precise and reliable measurement methods are therefore essential to guide clinical decision making. Yet, previous studies have indicated substantial differences in Lp(a) values as measured by various assays and among different racial groups (3, 4). An important limitation in the inconsistency of measurements is the fact

This work was supported by the EPIC-Norfolk Study and is funded by Cancer Research UK Grant 14136 and the Medical Research Council Grant G1000143. The funders had no role in the study design, data collection, analysis, decision to publish, or preparation of the manuscript. S. Tsimikas and J. L. Witztum are supported by National Institutes of Health Grants R01 HL119828, P01 HL088093, P01 HL055798, R01 HL106579, R01 HL078610, and R01 HL124174. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. Disclosures: S. Tsimikas and J. L. Witztum are coinventors and receive royalties from patents owned by the University of California, San Diego (UCSD) on oxidation-specific antibodies and of biomarkers related to oxidized lipoproteins. J. L. Witztum is a consultant to Ionis Pharmaceuticals and Intercept Pharmaceuticals. S. Tsimikas currently holds a dual appointment at UCSD and Ionis Pharmaceuticals. The other authors have no potential conflicts to declare.

Manuscript received 5 January 2016 and in revised form 23 January 2016.

Published, JLR Papers in Press, January 31, 2016
DOI 10.1194/jlr.P066258

Copyright © 2016 by the American Society for Biochemistry and Molecular Biology, Inc.

This article is available online at <http://www.jlr.org>

Abbreviations: CAD, coronary artery disease; CHD, coronary heart disease; CI, confidence interval; EAS, European Atherosclerosis Society; EPIC, European Prospective Investigation of Cancer; KIV₂, kringle domain IV type 2; Lp(a), lipoprotein (a); UCSD, University of California, San Diego.

¹Guest editor for this article was Alan M. Fogelman, David Geffen School of Medicine at UCLA.

²To whom corresponding should be addressed.
e-mail: stsimikas@ucsd.edu

that many assays are affected by the size of apo(a), the major protein component of Lp(a), and that antibodies and calibrators vary across platforms. Based on genetic variants of this protein, large interindividual differences in apo(a) isoform size exist.

Previous studies have attempted to elucidate the clinical relevance of the poor interassay agreement in terms of cardiovascular risk classification. Marcovina et al. (3) assessed the concordance in Lp(a) results among 22 different Lp(a) assays. Considerable heterogeneity was observed, in particular between apo(a) size-sensitive assays. The authors also speculated that this inaccuracy might translate into inaccurate risk assessment. A direct comparison of three Lp(a) assays in a subset of the Framingham Offspring cohort demonstrated large interassay differences in Lp(a) values and consequently in the 80th percentile cutoff values, which was largely determined by differences in apo(a) isoform size (4). However, the number of coronary artery disease (CAD) events in that study was limited. A recent meta-analysis evaluated the CVD predictive value between pooled isoform-sensitive and isoform-insensitive assays (5) and observed a substantial heterogeneity between the different studies. However, no formal assay comparisons were made, and assays were performed in different study populations, making it impossible to directly compare the predictive value of different assays.

Understanding appropriate assay and population thresholds for risk are essential as novel therapies, including antisense oligonucleotides, proprotein convertase subtilisin kexin type 9 inhibitors and cholesteryl ester transfer protein inhibitors, and mipomersen (6–10), lower Lp(a). Therefore, our objectives were 2-fold: first, to determine whether the population thresholds previously suggested for optimal levels apply broadly to other groups of patients and, second, to determine the concordance between a clinically available and commonly used immunoturbidimetric Lp(a) assay compared with a research-based ELISA in predicting CAD risk. We addressed this objective in a case-control study nested in the European Prospective Investigation of Cancer (EPIC)-Norfolk Prospective Population Study.

METHODS

Study design

The EPIC-Norfolk Prospective Population Study has been described in detail previously (11). In brief, the EPIC-Norfolk Prospective Population Study included 25,663 men and women aged between 45 and 79 years old and living in Norfolk, United Kingdom. The study was designed to determine the effects of diet and other lifestyle factors on the risk of developing cancer, and additional data were obtained to study determinants of other diseases. Participants were recruited using registers from general practices and were included after written invitation. All subjects have been flagged for death certification at the United Kingdom Office of National Statistics, with vital status ascertained for the entire cohort. In addition, all hospitalizations for study participants were recorded using their unique National Health Service number by data linkage with ENCORE, the East Norfolk Health

Authority database, which identifies all hospital contacts throughout England and Wales for Norfolk residents. Trained nosologists coded the underlying cause of hospital admission or death according to the 10th revision of the International Classification of Diseases (ICD-10). Fatal CAD during follow-up was defined as death with the underlying cause coded as ICD-10 codes I20 to I25. Nonfatal CAD was defined as hospitalization with the underlying cause coded as I20 to I25.

Participants

For this specific study, we analyzed the data of a case-control study originally described by Boekholdt et al. (12). Briefly, participants were marked as a case if they were apparently healthy at baseline and had died or been hospitalized with CAD as the underlying cause during follow-up. Controls were defined as study participants who were apparently healthy at baseline and did not develop any CVD during follow-up. In the original study design, we attempted to match two controls to each case, by sex, age (within 5 years), and date of baseline visit (within 3 months). In this study set, Lp(a) levels were measured in 2,160 participants at the University of California, San Diego (UCSD), as previously described (13). Several years later, it was decided to measure Lp(a) levels in the entire EPIC-Norfolk cohort at the University of Cambridge, depending on sample availability, as previously described (14). These included samples from the case-control study. Due to insufficient sample material, however, not all samples were measured with both assays. Therefore, the current analysis is based on the overlap between these two rounds of Lp(a) measurements.

Biochemical analysis

At the baseline study visit, blood was drawn from study participants, as previously described (11). Total cholesterol, HDL-cholesterol, and triglycerides were determined with the RA 1000 (Bayer Diagnostics, Basingstoke, United Kingdom). The Friedewald formula was used for the calculation of LDL-cholesterol levels. Later in time, Lp(a) levels were determined in nonfasting baseline samples that had been stored at -80°C .

In the Randox assay, Lp(a) levels were determined on a Olympus AU640 analyzer with an immunoturbidimetric method (Randox laboratories Ltd. Crumlin, County Antrim, United Kingdom) (14). This assay uses latex particles containing rabbit anti-human Lp(a) polyclonal antibody as a reagent (licensed from Denka Seiken Co. Ltd., Niigata, Japan) and Randox's own calibrators and controls from screened plasma donated by volunteers. The rabbit anti-human Lp(a) polyclonal antibody is technically isoform sensitive by virtue of the antisera binding to multiple sites of kringle domain IV type 2 (KIV₂) repeats, but the assay is theoretically made nearly isoform independent by the calibrator system. This assay format, like most commercial assays, binds to both free apo(a) and true Lp(a) [i.e., apo(a) covalently bound to apoB-100]; therefore, it is best described as measuring "total apo(a)" rather than "Lp(a)."

In the UCSD assay, designed and conducted at UCSD, Lp(a) levels were also determined with an Lp(a) assay designed by Tsimikas et al. (15), which incorporates the murine monoclonal antibody LPA4 with a chemiluminescent ELISA (13). LPA4 is a murine monoclonal IgG antibody to apo(a) that was generated by immunizing mice with the apo(a) sequence TRNYCRNPDAE-IRP. This sequence is present as one copy on KIV₅, KIV₇, and KIV₈ of apo(a) and does not cross-react with plasminogen. This assay is also a true "Lp(a)" assay as it uses a capture antibody to apoB-100 and detects apo(a) with biotinylated LPA4. Free apo(a) is not detected with this assay, but it is usually not present to any significant amount in humans in general populations. This assay has high sensitivity and a broad linear range from 0.5 to

180 mg/dl. This assay uses nine calibrators, ranging from 6 to 168 mg/dl to capture a wide range of values, identified from individual human plasma samples. The values assigned to the calibrators were validated by both the Diasorin (Stillwater, MN) and Technoclone (Vienna, Austria) calibrators. This assay correlates well with commercially available Lp(a) assays, has a coefficient of variability of 3–5%, and has been used in more than 20 studies and 20,000 patients (13, 16–19).

Statistical analysis

The current analysis is based on availability of both Lp(a) measurements in the cohort database. The selection of cases and controls is therefore based on the overlap between Lp(a) measurements performed in a nested case-control set as described above and the entire EPIC-Norfolk cohort. By virtue of this selection procedure, the original 1:2 matching by sex, age, and enrollment time was partially lost.

Baseline characteristics were calculated for cases and controls separately, stratified by sex. The correlation between Lp(a) levels as measured by the Randox and UCSD assays was quantified using Spearman correlation coefficients. Because the EAS Consensus Panel recommended using the 80th percentile as the cutoff value for an elevated Lp(a) level (2), we performed analyses using both sex-specific and pooled Lp(a) quintiles. In addition, because the 80th percentile in Danish populations is thought to correspond roughly to an Lp(a) level of 50 mg/dl, we also performed analyses in which participants were stratified using the Lp(a) threshold of 50 mg/dl. To assess the association between Lp(a) levels and the risk of developing CAD, logistic regression analyses were performed to determine the odds ratios and corresponding 95% confidence intervals (CIs) per Lp(a) quintile, using the lowest quintile as reference category. Furthermore, odds ratios were determined for participants with Lp(a) levels ≥ 50 mg/dl, using those < 50 mg/dl as reference category. Analyses were performed separately for men and women using sex-specific cutoffs, and also for men and women combined using pooled cutoffs. In addition, we assessed the concordance between the assays in classifying study participants as < 50 mg/dl versus ≥ 50 mg/dl. All statistical analyses were performed with SPSS statistical software (Version 21.0; IBM Corporation, Armonk, NY).

RESULTS

A total of 1,571 study participants had Lp(a) measurements done by both the Randox and UCSD assays. This set comprised 623 CAD cases and 948 matched controls. Baseline characteristics are shown in **Table 1**. As expected, cases had higher levels of LDL-cholesterol, higher blood pressure, and higher body mass index and were more likely to smoke and have diabetes mellitus, compared with controls. Median (interquartile range) Lp(a) levels measured by the Randox assay were 14.3 (7.2–35.9) mg/dl for male cases, 11.2 (6.1–23.2) mg/dl for male controls, 15.5 (8.5–37.9) mg/dl for female cases, and 11.4 (6.8–21.9) mg/dl for female controls. Median Lp(a) levels measured by the UCSD assay were 9.3 (6.8–23.9) mg/dl for male cases, 8.1 (6.4–13.4) mg/dl for male controls, 10.4 (7.4–29.8) mg/dl for female cases, and 8.5 (6.5–12.9) mg/dl for female controls.

Lp(a) levels as quantified by the Randox and UCSD assays were strongly correlated. Spearman's correlation coefficients for men, women, and sexes combined were 0.905,

0.915, and 0.909, respectively ($P < 0.001$ for each). The differences in Lp(a) levels measured by the Randox and UCSD assays were most prominent in the highest quintiles, with the Randox assay yielding higher Lp(a) levels than the UCSD assay, as shown in **Tables 2 and 3**. The 80th percentile thresholds were also different. The Randox assay yielded 80th percentile threshold values of 35 mg/dl, 37 mg/dl, and 36 mg/dl for men, women, and sexes combined, respectively. The corresponding 80th percentile thresholds according to the UCSD assay were 23 mg/dl, 26 mg/dl, and 24 mg/dl for men, women, and sexes combined, respectively. Importantly, with both assays, the 80th percentile thresholds were well below 50 mg/dl. Using the Randox assay, the distribution of cases versus controls among the pooled sexes ranged from 107/208 (34.0%) in the lowest quintile to 166/148 (52.9%) in the highest quintile. Using the UCSD assay the corresponding numbers were 102/212 (32.5%) in the lowest quintile and 166/148 (52.9%) in the highest quintile. In the group ≥ 50 mg/dl, the numbers of cases versus controls were 95/82 (55.2%) for the Randox assay and 53/34 (60.9%) for the UCSD assay.

Odds ratios for the risk of CAD by Lp(a) quintiles, as well as for the comparison ≥ 50 mg/dl versus < 50 mg/dl, are shown in **Table 4** and **Fig. 1**. Odds ratios for people in the top quintile (i.e., > 80 th percentile) compared with those in the lowest quintile were 2.18 (95% CI, 1.58–3.01) using the Randox assay and 2.35 (95% CI, 1.70–3.26) using the UCSD assay. Odds ratios for people with Lp(a) ≥ 50 mg/dl versus < 50 mg/dl were 2.29 (95% CI, 1.56–3.36) for the Randox assay and 2.85 (95% CI, 1.66–4.90) for the UCSD assay.

The concordance/discordance between the two assays in classifying study participants according to an Lp(a) level above or below 50 mg/dl is shown in **Table 5**. A total of 1,465 study participants had concordant results for both assays. Discordant results were observed in 106 study participants (6.9% of total subjects), with 8 having Lp(a) < 50 mg/dl on the Randox assay but Lp(a) ≥ 50 mg/dl on the UCSD assay, whereas 98 having Lp(a) ≥ 50 mg/dl on the Randox assay but Lp(a) < 50 mg/dl on the UCSD assay.

A Bland-Altman analysis was performed to determine the level of agreement between the UCSD and the Randox assays (**Fig. 2**). The mean average was -5.9 mg/dl, the lower 95% limit of agreement was -16.3 mg/dl, and the upper 95% limit of agreement was -28.2 mg/dl. At low average concentrations, the level of agreement was good, but with increasing concentrations, the level of agreement diminished.

DISCUSSION

This study evaluated population and assay thresholds for the predictive value of Lp(a) for the risk of CAD with two different assays measured in the same subjects. Interestingly, nearly identical predictive value for identifying subjects with or without CAD was evident, but this was true when examined in the context of the whole population studied

TABLE 1. Baseline characteristics

| | Men | | Women | |
|------------------------------------|-----------------|-----------------|-----------------|-----------------|
| | Controls | Cases | Controls | Cases |
| | n = 592 | n = 380 | n = 356 | n = 243 |
| Age, years | 63.8 ± 8.0 | 65.0 ± 8.0 | 66.7 ± 6.9 | 67.7 ± 6.6 |
| Body mass index, kg/m ² | 26.2 ± 2.8 | 27.1 ± 3.2 | 26.1 ± 4.2 | 26.8 ± 4.3 |
| Current smoker | 47 (8.0%) | 55 (14.5%) | 23 (6.5%) | 43 (18.0%) |
| Diabetes mellitus | 18 (3.0%) | 40 (10.5%) | 8 (2.2%) | 16 (6.6%) |
| Systolic blood pressure, mmHg | 137 ± 17 | 144 ± 18 | 137 ± 18 | 142 ± 20 |
| Diastolic blood pressure, mmHg | 83 ± 10 | 87 ± 12 | 81 ± 11 | 84 ± 12 |
| Total cholesterol, mM | 6.0 ± 1.1 | 6.3 ± 1.1 | 6.7 ± 1.2 | 6.9 ± 1.3 |
| LDL-cholesterol, mM | 3.9 ± 0.9 | 4.2 ± 1.0 | 4.3 ± 1.1 | 4.5 ± 1.1 |
| HDL-cholesterol, mM | 1.3 ± 0.3 | 1.2 ± 0.3 | 1.6 ± 0.4 | 1.5 ± 0.4 |
| Triglycerides, mM | 1.9 ± 0.9 | 2.1 ± 1.0 | 1.8 ± 0.9 | 2.1 ± 1.0 |
| Lp(a) Randox assay, mg/dl | 11.2 (6.1–23.2) | 14.3 (7.2–35.9) | 11.4 (6.8–21.9) | 15.5 (8.5–37.9) |
| Lp(a) UCSD assay, mg/dl | 8.1 (6.4–13.4) | 9.3 (6.8–23.9) | 8.5 (6.5–12.9) | 10.4 (7.4–29.8) |

Data are presented as mean ± SD for continuous variables with a normal distribution, median (interquartile range) for continuous variables with a nonnormal distribution, and number (percentage) for categorical variables.

for the given assay (e.g., in terms of quintiles), but not when comparing absolute Lp(a) levels and 80th percentile cutoff values. Finally, for both assays, the 80th percentile cutoffs were significantly lower than 50 mg/dl, the EAS proposed threshold, which was based on the Copenhagen Genera Population Study (2, 20, 21). This threshold was based on prevalence of Lp(a) values in the general population and not necessarily based on when risk of Lp(a) begins. Epidemiological and genetic studies suggest the risk thresholds start at 25–30 mg/dl in primary care populations (1, 14, 20), but >50 mg/dl in secondary prevention populations that have been treated with several secondary prevention measures such as aspirin, clopidogrel, statins, and antihypertensive medications (22, 23). Our findings suggest that the thresholds for determining what is a high level and who is at risk should be reported as assay-specific thresholds until assays from all manufacturers are sufficiently standardized and each assay provides the same absolute values for a given plasma sample. Preferably, a set of

internationally accepted standards and calibrators should be agreed on and implemented across all assay platforms.

Lp(a) consists of an LDL-like particle, in which apoB-100 is covalently bound to apo(a). Apo(a) consists of several kringle domains, in which the number of KIV₂ repeats is the dominant size-determining domain of apo(a); each apo(a) isoform can contain from 3 up to >40 KIV₂ repeats (24, 25). The number of KIV₂ repeats is inversely related with the plasma level of Lp(a) and explains 25–50% of plasma Lp(a) levels (20, 26). Additional genetic determinants of variability are present in the LPA gene and include regulatory elements and single nucleotide polymorphisms that mediate either high or low Lp(a) levels (27–31). These unique properties of Lp(a) greatly contribute to the difficulty of establishing common standards for its clinical measurement.

Lp(a) measurement methods are based on a variety of techniques, including immunonephelometry, immunoturbidometry, and ELISAs (32). In contrast to other

TABLE 2. Distribution of coronary heart disease (CHD) cases and controls by Lp(a) categories, UCSD assay

| | UCSD Assay | | | | | | |
|---------------------|-----------------|-----------|-----------|------------|-------------|-------------|-------------|
| | Lp(a) Quintiles | | | | | ≥30.0 mg/dl | ≥50.0 mg/dl |
| | 1 | 2 | 3 | 4 | 5 | | |
| Men | | | | | | | |
| Lp(a) range, mg/dl | 3.4–6.2 | 6.2–7.6 | 7.6–9.8 | 9.8–23.1 | 23.1–89.5 | 30.4–89.5 | 50.0–89.5 |
| Lp(a) mean, mg/dl | 5.5 ± 0.5 | 6.8 ± 0.4 | 8.5 ± 0.6 | 13.8 ± 3.9 | 41.8 ± 15.0 | 47.1 ± 13.7 | 63.6 ± 10.0 |
| Cases/controls, n/n | 66/128 | 63/132 | 73/121 | 82/113 | 96/98 | 71/74 | 29/19 |
| Cases, % of total | 34 | 32.3 | 37.6 | 42.1 | 49.5 | 49 | 60.4 |
| Total, n | 194 | 195 | 194 | 195 | 194 | 145 | 48 |
| Women | | | | | | | |
| Lp(a) range, mg/dl | 4.6–6.4 | 6.4–8.0 | 8.0–10.6 | 10.6–25.7 | 25.8–99.7 | 30.1–99.7 | 50.4–99.7 |
| Lp(a) mean, mg/dl | 5.7 ± 0.4 | 7.2 ± 0.4 | 9.2 ± 0.7 | 14.6 ± 3.9 | 45.5 ± 15.8 | 48.1 ± 15.4 | 64.9 ± 11.7 |
| Cases/controls, n/n | 84/35 | 44/76 | 45/75 | 50/70 | 69/51 | 60/44 | 24/15 |
| Cases, % of total | 29.4 | 36.7 | 37.5 | 41.7 | 57.5 | 57.7 | 61.5 |
| Total, n | 119 | 120 | 120 | 120 | 120 | 104 | 39 |
| Combined | | | | | | | |
| Lp(a) range, mg/dl | 3.4–6.3 | 6.3–7.7 | 7.7–10.2 | 10.1–23.8 | 24.0–99.7 | 30.1–99.7 | 50.0–99.7 |
| Lp(a) mean, mg/dl | 5.6 ± 0.5 | 7.0 ± 0.4 | 8.8 ± 0.6 | 14.1 ± 3.9 | 43.2 ± 15.0 | 47.5 ± 14.4 | 64.2 ± 10.7 |
| Cases/controls, n/n | 102/212 | 109/205 | 116/199 | 130/184 | 166/148 | 131/118 | 53/34 |
| Cases, % of total | 32.5 | 34.7 | 36.8 | 41.4 | 52.9 | 52.6 | 60.9 |
| Total, n | 314 | 314 | 315 | 314 | 314 | 249 | 87 |

Data are presented as mean ± SD, number or percentage. For the categories “men” and “women,” sex-specific quintiles were calculated. For the category “combined,” pooled quintiles were calculated. Lp(a) ranges for each quintile represent the approximate cutoff values.

TABLE 3. Distribution of CHD cases and controls by Lp(a) categories, Randox assay

| | Randox Assay | | | | | | |
|---------------------|-----------------|-----------|------------|------------|-------------|-------------|-------------|
| | Lp(a) Quintiles | | | | | ≥30.0 mg/dl | ≥50.0 mg/dl |
| | 1 | 2 | 3 | 4 | 5 | | |
| Men | | | | | | | |
| Lp(a) range, mg/dl | 2.2–5.5 | 5.5–9.5 | 9.5–15.9 | 16.0–34.8 | 34.9–162.3 | 30.2–162.3 | 50.2–162.3 |
| Lp(a) mean, mg/dl | 3.8 ± 0.8 | 7.4 ± 1.1 | 12.2 ± 1.8 | 23.1 ± 5.3 | 58.3 ± 23.5 | 55.2 ± 23.6 | 72.9 ± 23.6 |
| Cases/controls, n/n | 65/128 | 70/125 | 65/130 | 83/112 | 97/97 | 108/113 | 52/52 |
| Cases, % of total | 33.7 | 35.9 | 33.3 | 42.6 | 50 | 48.9 | 50 |
| Total, n | 193 | 195 | 195 | 195 | 194 | 221 | 104 |
| Women | | | | | | | |
| Lp(a) range, mg/dl | 2.2–6.4 | 6.4–10.3 | 10.3–16.6 | 16.7–36.8 | 37.0–143.5 | 30.2–143.5 | 50.1–143.5 |
| Lp(a) mean, mg/dl | 4.4 ± 1.1 | 8.3 ± 1.1 | 12.9 ± 1.8 | 24.8 ± 6.1 | 63.2 ± 24.5 | 57.8 ± 24.8 | 75.6 ± 24.0 |
| Cases/controls, n/n | 38/81 | 43/77 | 46/74 | 51/69 | 65/55 | 82/65 | 43/30 |
| Cases, % of total | 31.9 | 35.8 | 38.3 | 42.5 | 54.2 | 55.8 | 61.4 |
| Total, n | 119 | 120 | 120 | 120 | 120 | 147 | 73 |
| Combined | | | | | | | |
| Lp(a) range, mg/dl | 2.2–5.8 | 5.9–9.8 | 9.8–16.2 | 16.2–35.4 | 35.6–162.3 | 30.2–162.3 | 50.1–162.3 |
| Lp(a) mean, mg/dl | 4.0 ± 0.8 | 7.8 ± 1.1 | 12.5 ± 1.8 | 23.8 ± 5.3 | 60.2 ± 23.5 | 56.2 ± 24.1 | 74.0 ± 23.7 |
| Cases/controls, n/n | 107/208 | 109/205 | 111/203 | 130/184 | 166/148 | 190/178 | 95/82 |
| Cases, % of total | 34 | 34.7 | 35.4 | 41.4 | 52.9 | 51.6 | 55.2 |
| Total, n | 315 | 314 | 314 | 314 | 314 | 368 | 177 |

Data are presented as mean ± SD, number or percentage. For the categories “men” and “women,” sex-specific quintiles were calculated. For the category “combined,” pooled quintiles were calculated. Lp(a) ranges for each quintile represent the approximate cutoff values.

protein quantification assays, Lp(a) assays are notoriously challenging because of the large variation in size and structure of the apo(a) protein size, which is mediated by the allelic heterogeneity in the number of KIV₂ repeats. Additionally, each individual generally has two expressed alleles that usually code for two different-sized apo(a) proteins in plasma. More than 40 different-sized isoforms of apo(a) have been reported, and ~80% of individuals have two different-sized apo(a) isoforms in plasma (29). Furthermore, although the molecular weight of apoB-100 is fixed, differences besides variability of KIV₂ repeats may exist in additional components of Lp(a), such as the carbohydrate content on apo(a) and the lipid composition of the LDL, which may further complicate the accurate quantification of Lp(a) levels by mass (33). Other aspects that impact precision and reproducibility are the use of

different detection antibodies and, importantly, the lack of common standards and particularly uniform calibrators.

Almost all commercially available methods use polyclonal antibodies, which are, by definition, isoform dependent because polyclonal antibody preparations contain heterogeneous mixture of antibodies that bind to different sites on apo(a) protein. Because KIV₂ repeats are generally the most prevalent kringles, it is expected that a majority of binding sites of such polyclonal antibodies will be at KIV₂ repeats. The only isoform-independent antibody, as defined by virtue of not binding to KIV₂ repeats, is monoclonal antibody a6 from the Northwest Lipid Metabolism and Diabetes Research Laboratories that binds to KIV₉. It is also important to emphasize that most commercially available assays measure “total apo(a)” rather than Lp(a). Because all polyclonal antibodies will likely bind to KIV₂,

TABLE 4. Odds ratios for CHD risk by Lp(a) categories

| | Lp(a) Quintiles | | | | | | |
|-----------------|-----------------|---------------------|---------------------|---------------------|---------------------|-------------|---------------------|
| | 1 | 2 | 3 | 4 | 5 | <50.0 mg/dl | ≥50.0 mg/dl |
| | Men | | | | | | |
| Randox assay | 1.00 | 1.10 (0.72–1.67) | 0.97 (0.64–1.49) | 1.45 (0.96–2.19) | 1.99 (1.32–3.01) | 1.00 | 1.74 (1.15–2.62) |
| UCSD assay | 1.00 | 0.93 (0.61–1.42) | 1.17 (0.77–1.78) | 1.41 (0.93–2.13) | 1.95 (1.29–2.94) | 1.00 | 2.55 (1.41–4.63) |
| Women | | | | | | | |
| Randox assay | 1.00 | 1.18 (0.69–2.01) | 1.30 (0.76–2.21) | 1.52 (0.89–2.58) | 2.48 (1.46–4.21) | 1.00 | 2.36 (1.43–3.89) |
| UCSD assay | 1.00 | 1.36 (0.79–2.34) | 1.39 (0.81–2.39) | 1.63 (0.95–2.79) | 3.21 (1.88–5.49) | 1.00 | 2.50 (1.28–4.87) |
| Combined | | | | | | | |
| Randox assay | 1.00 | 1.03 (0.74–1.43) | 1.05 (0.75–1.46) | 1.35 (0.98–1.87) | 2.18 (1.58–3.01) | 1.00 | 1.97 (1.43–2.70) |
| UCSD assay | 1.00 | 1.10 (0.79–1.53) | 1.20 (0.86–1.67) | 1.45 (1.04–2.01) | 2.35 (1.70–3.26) | 1.00 | 2.53 (1.62–3.95) |

Data are presented as odds ratios with corresponding 95% CI limits. For the categories men and women, sex-specific, age-adjusted odds ratios were calculated, with the Lp(a) cutoff values as presented in Tables 2 and 3. For the category combined, sex- and age-adjusted odds ratios were calculated, with the Lp(a) cutoff values as presented in Tables 2 and 3. Odds ratios per quintile with the lowest quintile as reference category. In addition, an odds ratio is presented for people with Lp(a) >50 mg/dl, using those with Lp(a) <50 mg/dl as reference category.

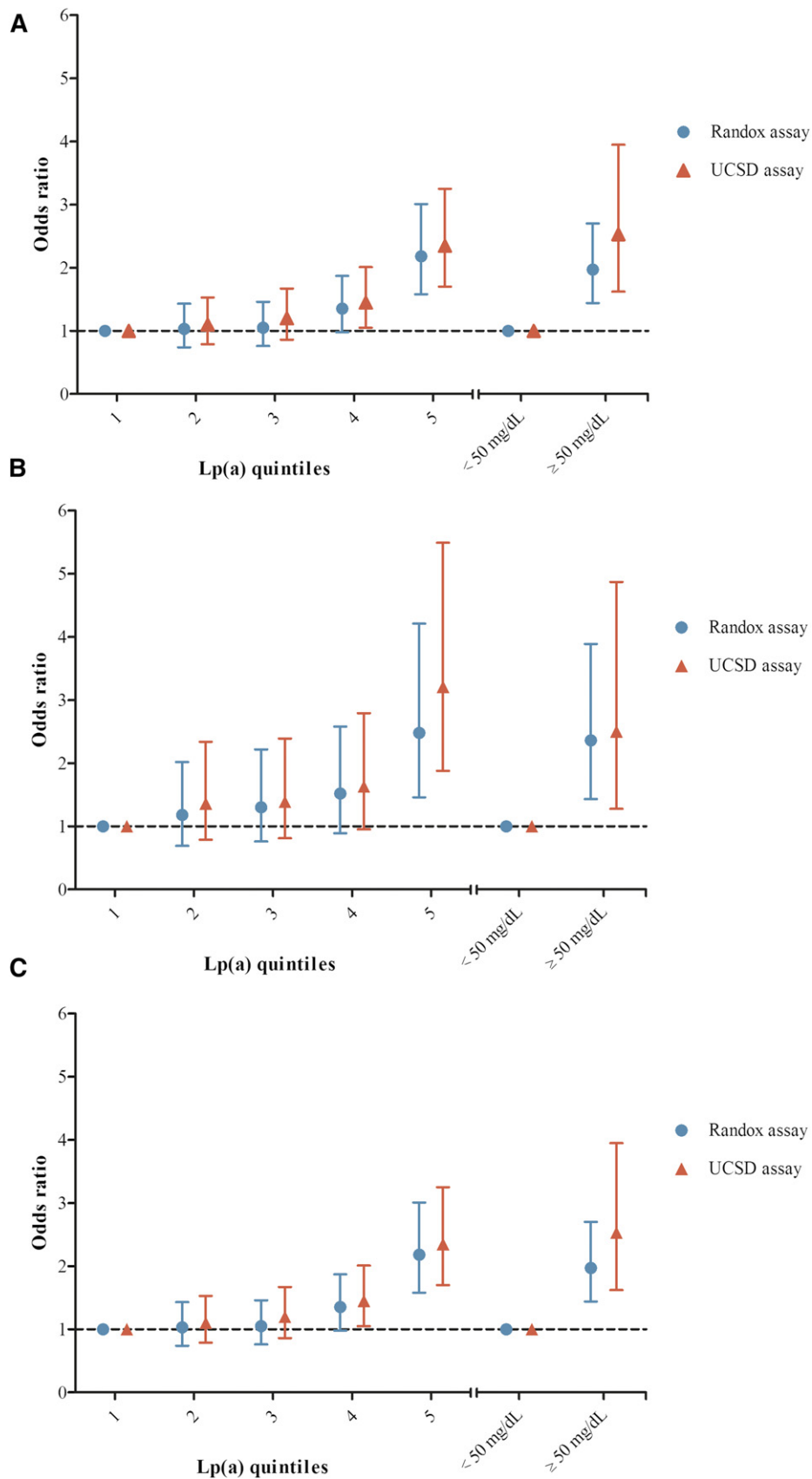


Fig. 1. Odds ratios for CHD risk by Lp(a) categories for men (A), women (B), and both sexes combined (C). Data are presented as sex-specific, age-adjusted odds ratios with corresponding 95% CI limits. Odds ratios per quintile with the lowest quintile as reference category. In addition, an odds ratio is presented for people with Lp(a) >50 mg/dl, using those with Lp(a) <50 mg/dl as reference category.

TABLE 5. Number of study participants with an Lp(a) level <50 mg/dl and ≥50 mg/dl per assay

| UCSD Assay | Lp(a) | Randox Assay | |
|------------|-------------|--------------|-------------|
| | | <50.0 mg/dl | ≥50.0 mg/dl |
| Men | <50.0 mg/dl | 861 | 63 |
| | ≥50.0 mg/dl | 7 | 41 |
| Women | <50.0 mg/dl | 525 | 35 |
| | ≥50.0 mg/dl | 1 | 38 |
| Combined | <50.0 mg/dl | 1,386 | 98 |
| | ≥50.0 mg/dl | 8 | 79 |

Data are presented as numbers.

ideally monoclonal antibodies not binding KIV₂ should be used to avoid the issue of isoform sensitivity. Although the issue of isoform sensitivity has been addressed to some extent by careful selection of calibrators, it is quite likely that use of polyclonal antibodies will always have some isoform sensitivity if carefully analyzed.

Although manufacturers do not usually publically report the process of generating appropriate calibrators, they generally use pooled plasma samples from many donors to isolate Lp(a). Often, such calibrator sets result from serial dilutions of pooled high Lp(a) plasma but can also be separate pools of plasma that have mean values ranging from low to high. It is important that the calibrators used reflect the presence of the whole range of different-sized alleles both between and within individuals, and that such alleles are present in the sample being measured. A further disadvantage of using human pooled plasma for calibrators is that each batch changes according to the availability of donors over months and years, and therefore, there may be variation each time a new batch is made. This issue will become increasingly important as the measurement of Lp(a) is predicted to increase substantially with available therapies to lower levels and the measurement of Lp(a) levels increases globally. Ideally,

calibrators should be linked to the reference material from the World Health Organization to ensure relative isoform independence.

To address the limitation of interassay variation, many investigators suggest the use of apo(a) isoform-independent assays corrected with international secondary Lp(a) references. Also the proposal has been made to determine the concentration of Lp(a) as particle number reported in nanomoles per liter, preferably using the reference standard of the International Federation of Clinical Chemistry as a way to minimize the issues related to variability in Lp(a) mass (33, 34). This World Health Organization-approved standard, with an Lp(a) concentration of 107 nM and 21 KIV repeats, ensures a fixed and apo(a) isoform-independent measurement of the molar concentrations of Lp(a) (3). Although one can roughly convert milligrams per deciliter to nanomoles per liter with a ratio of ~2.4, in reality, this is not appropriate and should not be carried out as it depends on knowing the underlying isoform numbers as well as having a fixed concentration of the lipid and carbohydrate content of the Lp(a), which are usually not known (35). Ultimately, the use of molar-based measures coupled with advances in appropriate gold-standard calibrators without the need of pooled plasma

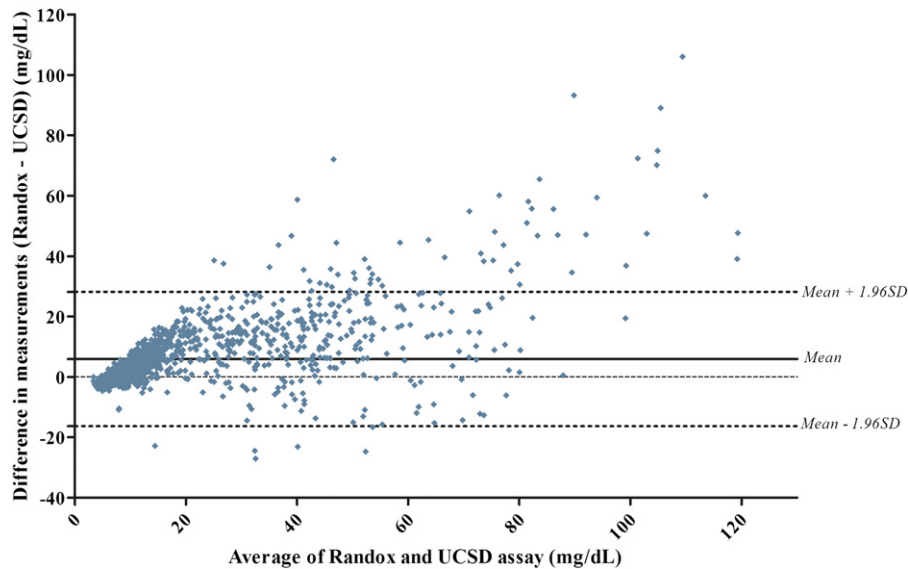



Fig. 2. Bland-Altman analysis of the average of the UCSD and Randox assays plotted against the mean difference. Data are presented as difference in measurements (Randox – UCSD) in mg/dl, mean, lower 95% limit of agreement (mean – 1.96 SD), and upper 95% limit of agreement (mean + 1.96 SD). Average of Randox and UCSD assays were calculated.

will pave the way to fully standardize these assays, improve clinical decision making, and optimize clinical trial design with new therapeutic approaches.

As we have previously shown in the EPIC-Norfolk cohort, high Lp(a) levels were strongly associated with an increased risk of CAD (13, 14). These observations are consistent with the EAS consensus statement and multiple studies and meta-analyses consistently demonstrating a curvilinear correlation between Lp(a) levels and increased risk of CVD (2, 5, 13). In the current study, independent of the type of assay or the sex of the study participants, both Lp(a) levels in excess of 50 mg/dl or above the 5th quintile (or 80th percentile) were correlated with increased CAD risk. The 80th percentile cutoff values, in a population enriched in CAD cases, of 36 mg/dl for the Randox assay and 24 mg/dl for the UCSD assay were considerably lower than the EAS proposed threshold of 50 mg/dl but are consistent with epidemiological and trial data (2, 5, 20, 21). This discrepancy is relevant because clinical decision making is suggested to be made on the 50 mg/dl threshold in Europe, although most US laboratories use >30 mg/dl as elevated risk (36). As shown by Mendelian randomization studies at the primary care level (20), that CVD risk associated with Lp(a) begins at much lower levels than 50 mg/dl. Another way to bypass specific thresholds is to determine assay-specific 80th percentile thresholds. In this way, the effect of interassay variation is minimized because each assay has its own cutoff value. For this to work, it is vital to determine Lp(a) levels of a broad range of persons. Although less favorable than a method based on international standards and calibrators, it can be a good interim solution. Finally, it was recently shown in the MESA study that race-specific levels of Lp(a) may also need to be instituted due to differences in the thresholds and predictive values for CHD across races (37). However, this was limited by a low number of cardiovascular events and wide CIs in the point estimates, suggesting a need for confirmation of these findings. Future studies, as well as clinical trials with novel drugs (6–10), will have to take into account what the appropriate risk threshold is to test the hypothesis that Lp(a) lowering prevents CVD events.

Limitations of this study are that it only included one epidemiological cohort, which was primarily Caucasian, and also only compared two assays. Further studies comparing a variety of assays in diverse populations will be needed to confirm these results.

In conclusion, these results demonstrate that lower cutoff values of Lp(a) could be clinically significant and the use of different assays could have large impact on clinical decision making. With the arrival of potent drugs to lower Lp(a) to “normal” levels, full standardization of assay methodologies will be needed to ultimately optimize clinical decision making for the estimated >1 billion people with potentially atherogenic Lp(a) levels (38). 

The authors wish to thank the participants and staff of the EPIC-Norfolk Prospective Population Study.

- Erqou, S., S. Kaptoge, P. L. Perry, E. Di Angelantonio, A. Thompson, I. R. White, S. M. Marcovina, R. Collins, S. G. Thompson, and J. Danesh. 2009. Lipoprotein(a) concentration and the risk of coronary heart disease, stroke, and nonvascular mortality. *J. Am. Med. Assoc.* **302**: 412–423.
- Nordstgaard, B. G., M. J. Chapman, K. Ray, J. Boren, F. Andreotti, G. F. Watts, H. Ginsberg, P. Amarengo, A. Catapano, O. S. Descamps, et al.; European Atherosclerosis Society Consensus Panel. 2010. Lipoprotein(a) as a cardiovascular risk factor: current status. *Eur. Heart J.* **31**: 2844–2853.
- Marcovina, S. M., J. J. Albers, A. M. Scanu, H. Kennedy, F. Giaculli, K. Berg, R. Couderc, F. Dati, N. Rifai, I. Sakurabayashi, et al. 2000. Use of a reference material proposed by the International Federation of Clinical Chemistry and Laboratory Medicine to evaluate analytical methods for the determination of plasma lipoprotein(a). *Clin. Chem.* **46**: 1956–1967.
- Lamon-Fava, S., S. M. Marcovina, J. J. Albers, H. Kennedy, C. Deluca, C. C. White, L. A. Cupples, J. R. McNamara, L. J. Seman, V. Bongard, et al. 2011. Lipoprotein(a) levels, apo(a) isoform size, and coronary heart disease risk in the Framingham Offspring Study. *J. Lipid Res.* **52**: 1181–1187.
- Erqou, S., A. Thompson, E. Di Angelantonio, D. Saleheen, S. Kaptoge, S. Marcovina, and J. Danesh. 2010. Apolipoprotein(a) isoforms and the risk of vascular disease: systematic review of 40 studies involving 58,000 participants. *J. Am. Coll. Cardiol.* **55**: 2160–2167.
- Tsimikas, S., N. J. Viney, S. G. Hughes, W. Singleton, M. Graham, B. F. Baker, J. L. Burke, Q. Yang, S. M. Marcovina, R. S. Geary, et al. 2015. Antisense therapy targeting apolipoprotein(a): a randomised, double-blind, placebo-controlled phase 1 study. *Lancet.* **386**: 1472–1483.
- Gaudet, D., D. J. Kereiakes, J. M. McKenney, E. M. Roth, C. Hanotin, D. Gipe, Y. Du, A. C. Ferrand, H. N. Ginsberg, and E. A. Stein. 2014. Effect of alirocumab, a monoclonal proprotein convertase subtilisin/kexin 9 antibody, on lipoprotein(a) concentrations (a pooled analysis of 150 mg every two weeks dosing from phase 2 trials). *Am. J. Cardiol.* **114**: 711–715.
- Raal, F. J., R. P. Giugliano, M. S. Sabatine, M. J. Koren, G. Langslet, H. Bays, D. Blom, M. Eriksson, R. Dent, S. M. Wasserman, et al. 2014. Reduction in lipoprotein(a) with PCSK9 monoclonal antibody evolocumab (AMG 145): a pooled analysis of more than 1,300 patients in 4 phase II trials. *J. Am. Coll. Cardiol.* **63**: 1278–1288.
- Cannon, C. P., S. Shah, H. M. Dansky, M. Davidson, E. A. Brinton, A. M. Gotto, M. Stepanavage, S. X. Liu, P. Gibbons, T. B. Ashraf, et al.; Determining the Efficacy and Tolerability Investigators. 2010. Safety of anacetrapib in patients with or at high risk for coronary heart disease. *N. Engl. J. Med.* **363**: 2406–2415.
- Santos, R. D., F. J. Raal, A. L. Catapano, J. L. Witztum, E. Steinhagen-Thiessen, and S. Tsimikas. 2015. Mipomersen, an antisense oligonucleotide to apolipoprotein B-100, reduces lipoprotein(a) in various populations with hypercholesterolemia: results of 4 phase III trials. *Arterioscler. Thromb. Vasc. Biol.* **35**: 689–699.
- Day, N., S. Oakes, R. Luben, K. T. Khaw, S. Bingham, A. Welch, and N. Wareham. 1999. EPIC-Norfolk: study design and characteristics of the cohort. European Prospective Investigation of Cancer. *Br. J. Cancer.* **80** (Suppl. 1): 95–103.
- Boekholdt, S. M., R. J. Peters, N. E. Day, R. Luben, S. A. Bingham, N. J. Wareham, C. E. Hack, P. H. Reitsma, and K. T. Khaw. 2004. Macrophage migration inhibitory factor and the risk of myocardial infarction or death due to coronary artery disease in adults without prior myocardial infarction or stroke: the EPIC-Norfolk Prospective Population study. *Am. J. Med.* **117**: 390–397.
- Tsimikas, S., Z. Mallat, P. J. Talmud, J. J. Kastelein, N. J. Wareham, M. S. Sandhu, E. R. Miller, J. Benessiano, A. Tedgui, J. L. Witztum, et al. 2010. Oxidation-specific biomarkers, lipoprotein(a), and risk of fatal and nonfatal coronary events. *J. Am. Coll. Cardiol.* **56**: 946–955.
- Gurdasani, D., B. Sjouke, S. Tsimikas, G. K. Hovingh, R. N. Luben, N. W. Wainwright, C. Pomilla, N. J. Wareham, K. T. Khaw, S. M. Boekholdt, et al. 2012. Lipoprotein(a) and risk of coronary, cerebrovascular, and peripheral artery disease: the EPIC-Norfolk Prospective Population Study. *Arterioscler. Thromb. Vasc. Biol.* **32**: 3058–3065.
- Tsimikas, S., H. K. Lau, K. R. Han, B. Shortal, E. R. Miller, A. Segev, L. K. Curtiss, J. L. Witztum, and B. H. Strauss. 2004. Percutaneous

- coronary intervention results in acute increases in oxidized phospholipids and lipoprotein(a): short-term and long-term immunologic responses to oxidized low-density lipoprotein. *Circulation*. **109**: 3164–3170.
16. Taleb, A., J. L. Witztum, and S. Tsimikas. 2011. Oxidized phospholipids on apoB-100-containing lipoproteins: a biomarker predicting cardiovascular disease and cardiovascular events. *Biomarkers Med*. **5**: 673–694.
 17. Tsimikas, S., J. L. Witztum, E. R. Miller, W. J. Sasiela, M. Szarek, A. G. Olsson, and G. G. Schwartz. 2004. High-dose atorvastatin reduces total plasma levels of oxidized phospholipids and immune complexes present on apolipoprotein B-100 in patients with acute coronary syndromes in the MIRACL trial. *Circulation*. **110**: 1406–1412.
 18. Willeit, P., S. Kiechl, F. Kronenberg, J. L. Witztum, P. Santer, M. Mayr, Q. Xu, A. Mayr, J. Willeit, and S. Tsimikas. 2014. Discrimination and net reclassification of cardiovascular risk with lipoprotein(a): prospective 15-year outcomes in the Bruneck Study. *J. Am. Coll. Cardiol*. **64**: 851–860.
 19. Leibundgut, G., C. Scipione, H. Yin, M. Schneider, M. B. Boffa, S. Green, X. Yang, E. A. Dennis, J. L. Witztum, M. L. Koschinsky, et al. 2013. Determinants of binding of oxidized phospholipids on apolipoprotein (a) and lipoprotein (a). *J. Lipid Res*. **54**: 2815–2830.
 20. Kamstrup, P. R., M. Benn, A. Tybjaerg-Hansen, and B. G. Nordestgaard. 2008. Extreme lipoprotein(a) levels and risk of myocardial infarction in the general population: the Copenhagen City Heart Study. *Circulation*. **117**: 176–184.
 21. Kamstrup, P. R., A. Tybjaerg-Hansen, and B. G. Nordestgaard. 2013. Extreme lipoprotein(a) levels and improved cardiovascular risk prediction. *J. Am. Coll. Cardiol*. **61**: 1146–1156.
 22. Albers, J. J., A. Slee, K. D. O'Brien, J. G. Robinson, M. L. Kashyap, P. O. Kwiterovich, Jr., P. Xu, and S. M. Marcovina. 2013. Relationship of apolipoproteins A-I and B, and lipoprotein(a) to cardiovascular outcomes: the AIM-HIGH trial (Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High Triglyceride and Impact on Global Health Outcomes). *J. Am. Coll. Cardiol*. **62**: 1575–1579.
 23. Nestel, P. J., E. H. Barnes, A. M. Tonkin, J. Simes, M. Fournier, H. D. White, D. M. Colquhoun, S. Blankenberg, and D. R. Sullivan. 2013. Plasma lipoprotein(a) concentration predicts future coronary and cardiovascular events in patients with stable coronary heart disease. *Arterioscler. Thromb. Vasc. Biol*. **33**: 2902–2908.
 24. Kraft, H. G., C. Sandholzer, H. J. Menzel, and G. Utermann. 1992. Apolipoprotein (a) alleles determine lipoprotein (a) particle density and concentration in plasma. *Arterioscler. Thromb*. **12**: 302–306.
 25. Marcovina, S. M., H. H. Hobbs, and J. J. Albers. 1996. Relation between number of apolipoprotein(a) kringle 4 repeats and mobility of isoforms in agarose gel: basis for a standardized isoform nomenclature. *Clin. Chem*. **42**: 436–439.
 26. Clarke, R., J. F. Peden, J. C. Hopewell, T. Kyriakou, A. Goel, S. C. Heath, S. Parish, S. Barlera, M. G. Franzosi, S. Rust, et al.; PROCARDIS Consortium. 2009. Genetic variants associated with Lp(a) lipoprotein level and coronary disease. *N. Engl. J. Med*. **361**: 2518–2528.
 27. Luke, M. M., J. P. Kane, D. M. Liu, C. M. Rowland, D. Shiffman, J. Cassano, J. J. Catanese, C. R. Pullinger, D. U. Leong, A. R. Arellano, et al. 2007. A polymorphism in the protease-like domain of apolipoprotein(a) is associated with severe coronary artery disease. *Arterioscler. Thromb. Vasc. Biol*. **27**: 2030–2036.
 28. Chasman, D. I., D. Shiffman, R. Y. Zee, J. Z. Louie, M. M. Luke, C. M. Rowland, J. J. Catanese, J. E. Buring, J. J. Devlin, and P. M. Ridker. 2009. Polymorphism in the apolipoprotein(a) gene, plasma lipoprotein(a), cardiovascular disease, and low-dose aspirin therapy. *Atherosclerosis*. **203**: 371–376.
 29. Boerwinkle, E., C. C. Leffert, J. Lin, C. Lackner, G. Chiesa, and H. H. Hobbs. 1992. Apolipoprotein(a) gene accounts for greater than 90% of the variation in plasma lipoprotein(a) concentrations. *J. Clin. Invest*. **90**: 52–60.
 30. Kronenberg, F., and G. Utermann. 2013. Lipoprotein(a): resurrected by genetics. *J. Intern. Med*. **273**: 6–30.
 31. Hoover-Plow, J., and M. Huang. 2013. Lipoprotein(a) metabolism: potential sites for therapeutic targets. *Metabolism*. **62**: 479–491.
 32. Marcovina, S. M., J. J. Albers, B. Gabel, M. L. Koschinsky, and V. P. Gaur. 1995. Effect of the number of apolipoprotein(a) kringle 4 domains on immunochemical measurements of lipoprotein(a). *Clin. Chem*. **41**: 246–255.
 33. Marcovina, S. M., M. L. Koschinsky, J. J. Albers, and S. Skarlatos. 2003. Report of the National Heart, Lung, and Blood Institute workshop on lipoprotein(a) and cardiovascular disease: recent advances and future directions. *Clin. Chem*. **49**: 1785–1796.
 34. Dati, F., J. R. Tate, S. M. Marcovina, A. Steinmetz; International Federation of Clinical Chemistry and Laboratory Medicine; IFCC Working Group for Lipoprotein(a) Assay Standardization. 2004. First WHO/IFCC international reference reagent for lipoprotein(a) for immunoassay – Lp(a) SRM 2B. *Clin. Chem. Lab. Med*. **42**: 670–676.
 35. Brown, W. V., C. M. Ballantyne, P. H. Jones, and S. Marcovina. 2010. Management of Lp(a). *J. Clin. Lipidol*. **4**: 240–247.
 36. Kostner, G. M., P. Avogaro, G. Cazzolato, E. Marth, G. Bittolo-Bon, and G. B. Quinici. 1981. Lipoprotein Lp(a) and the risk for myocardial infarction. *Atherosclerosis*. **38**: 51–61.
 37. Guan, W., J. Cao, B. T. Steffen, W. S. Post, J. H. Stein, M. C. Tattersall, J. D. Kaufman, J. P. McConnell, D. M. Hoefner, R. Warnick, et al. 2015. Race is a key variable in assigning lipoprotein(a) cutoff values for coronary heart disease risk assessment: the Multi-Ethnic Study of Atherosclerosis. *Arterioscler. Thromb. Vasc. Biol*. **35**: 996–1001.
 38. Tsimikas, S., and J. H. Hall. 2012. 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*. **60**: 716–721.