HHS Public Access

Author manuscript

Circulation. Author manuscript; available in PMC 2018 January 17.

Published in final edited form as:

Circulation. 2017 January 17; 135(3): 251–263. doi:10.1161/CIRCULATIONAHA.116.024611.

The *LPA* gene, ethnicity and cardiovascular events

Sang-Rok Lee, MD, PhD[1,2], **Anand Prasad, MD**[3], **Yun-Seok Choi, MD, PhD**[1,4], **Chao Xing, PhD**[5], **Paul Clopton, MS**[6], **Joseph L. Witztum, MD**[7], and **Sotirios Tsimikas, MD**[1]

[1]Division of Cardiovascular Diseases, Sulpizio Cardiovascular Center, Department of Medicine, University of California San Diego, La Jolla, CA

[2]Division of Cardiology, Chonbuk National University Hospital and Chonbuk School of Medicine, Jeonju, Korea

[3]Division of Cardiology, Department of Medicine, The University of Texas Health Science Center San Antonio, San Antonio, Texas

[4]Division of Cardiology, Department of Internal Medicine, College of Medicine, The Catholic University of Korea, Seoul, Korea

[5]Donald W. Reynolds Cardiovascular Clinical Research Center, the Eugene McDermott Center for Human Growth and Development, University of Texas Southwestern Medical Center at Dallas, Dallas, Texas

[6]Veterans Affairs Medical Center, San Diego

[7]Division of Endocrinology and Metabolism, University of California San Diego, La Jolla, CA

Abstract

Background—The relationship of *LPA* single nucleotide polymorphisms (SNPs), apolipoprotein(a) isoforms and lipoprotein(a) [Lp(a)] levels with major adverse cardiovascular (MACE) events in different ethnic groups is not well known.

Methods—*LPA* SNPs, apolipoprotein(a) isoforms, Lp(a) and oxidized phospholipids on apolipoprotein B-100 (OxPL-apoB) levels were measured in 1792 Black, 1030 White and 597 Hispanic subjects enrolled in the Dallas Heart Study. Their interdependent relationships and prospective association with MACE after median 9.5-year follow-up were determined.

Results—*LPA* SNP rs3798220 was most prevalent in Hispanics (42.38%), rs10455872 in Whites (14.27%) and rs9457951 in Blacks (32.927%). The correlation of each of these SNPs with the major apolipoprotein(a) isoform size was highly variable and in different directions among ethnic groups. In the entire cohort, Cox regression analysis with multivariable adjustment revealed that quartiles 4 of Lp(a) and OxPL-apoB were associated with hazard ratios (HR) (95% CI) for time to MACE of 2.35 (1.50-3.69), p<0.001) and 1.89 (1.26-2.84), p=0.003), respectively, versus quartile 1. Addition of the major apolipoprotein(a) isoform and the 3 *LPA* SNPs to these models attenuated

Corresponding Author: Sotirios Tsimikas, MD, Vascular Medicine Program, Sulpizio Cardiovascular Center, University of California San Diego, 9500 Gilman Drive, BSB 1080, La Jolla, CA 92093-0682. stsimikas@ucsd.edu, telephone 858-534-6109.

Disclosures: ST and JLW are co-inventors and receive royalties from patents owned by the University of California San Diego on oxidation-specific antibodies. ST has a dual appointment at UCSD and Ionis Pharmaceuticals. JLW is a consultant for Ionis Pharmaceuticals, Intercept, CymaBay and Prometheus. The other co-authors have no conflicts of interest.

the risk, but significance was maintained for both Lp(a) and OxPL-apoB. Evaluating specific ethnic groups, in Blacks Lp(a) was a positive predictor and the size of the major apolipoprotein(a) isoform and inverse predictor, in Whites the size of the major apolipoprotein(a) isoform was an inverse predictor and in Hispanics OxPL-apoB was a predictor of time to MACE.

Conclusion—The prevalence and association of *LPA* SNPs with size of apolipoprotein(a) isoforms, Lp(a) and OxPL-apoB levels are highly variable and ethnicity-specific. The relationship to MACE is best explained by elevated plasma Lp(a) or OxPL-apoB levels, despite significant ethnic differences in *LPA* genetic markers.

Keywords

lipoprotein(a); ethnicity; isoforms; single nucleotide polymorphisms; and cardiovascular events; oxidized phospholipids

Subject terms

Lipids and cholesterol; biomarkers; race and ethnicity; atherosclerosis

Introduction

Lipoprotein(a) [Lp(a)] is an independent, genetic and likely causal risk factor for myocardial infarction, stroke, peripheral arterial disease[1,2] and calcific aortic valve stenosis.[3,4] Circulating Lp(a) levels are largely genetically determined by a variety of differences in the *LPA* gene. There are several levels of *LPA* gene regulation that determine plasma Lp(a) levels, including biochemical influences on transcription factors, variations in *LPA* single nucleotide polymorphisms (SNPs) and the inter and intra individual heterogeneity in kringle IV type 2 (KIV_2) isoform repeats. Physiologic, dietary and environmental factors play a relatively minor role and can either lower or raise Lp(a) plasma levels.[5] Furthermore, it is well appreciated that significant ethnic differences exist in Lp(a) levels, with highest levels present in subjects of African descent and generally followed in decreasing order of lower levels in southern Asians, Whites, Hispanics and east Asians.[2,6,7]

LPA SNPs rs3798220 and rs10455872 in Whites and rs9457951 in Blacks have been shown to be associated with increased plasma Lp(a) levels In separate studies.[8,9] However, the independent association of *LPA* SNPs and apolipoprotein(a) isoforms with major adverse cardiovascular events (MACE) in populations as a whole and in different ethnic groups is not well established. The goals of this study were to evaluate these three major, well-defined *LPA* SNPs in their relationship to Lp(a) levels and apolipoprotein(a) isoforms in different ethnic groups and to assess their relationship of *LPA* SNPs to incident CVD events. This gap in the clinical database of Lp(a) is relevant in view of novel agents that can reduce Lp(a) levels, such as antibodies directed to proprotein convertase subtilisin kexin type 9 (PCSK9) to bind circulating PCSK9 generating immune complexes and preventing physiological action[10,11] and antisense oligonucleotides directed to apolipoprotein(a) messenger ribonucleic acid to reduce apolipoprotein(a) protein synthesis and thereby prevent assembly of Lp(a).[12] The Dallas Heart Study (DHS) provides a unique, prospective database with median 9.5 year follow-up to assess these relationships among White, Black and Hispanic

subjects to provide insights into the atherogenicity of Lp(a). We hypothesized that elevated circulating Lp(a) levels rather than ethnic *LPA* genetic differences would be the key determinant in predicting incident cardiovascular events.

Methods

Study subjects

The characteristics of the DHS subjects were previously described in detail.[7] The DHS is a multiethnic, probability-based sample of the Dallas county population in which Blacks were systematically over-sampled so they represented ~50% of the final sample size. In this study, 3,419 blood samples were available with complete data of *LPA* SNPs, apolipoprotein(a) isoforms, Lp(a) levels and accompanying ethnic, biomarker and major adverse cardiovascular event (MACE) data from 2001-2010 for subsequent analyses. The Institutional Review Board of the University of Texas Southwestern Medical Center approved the study, and all participants provided informed consent in accordance with institutional guidelines. The Human Subject protection program at University of California San Diego approved the measurement of oxidative biomarkers.

Determination of *LPA* SNPs

The *LPA* variants rs3798220, rs10455872, rs9457951, rs1801693, rs41272110 and G+1/inKIV-8A were genotyped using a TaqMan assay on a 7900HT Fast Real-Time PCR instrument (Applied Biosystems, Foster City, CA) at 50°C for 2 min, 95°C for 10 min, and then 40 cycles of 95°C for 15 seconds and 60°C for 1.5 minutes.

Determination of Lp(a) levels and apolipoprotein (a) isoforms

Measurement of Lp(a) levels was performed with a well-validated assay that is independent of apolipoprotein(a) isoform size and reported as in nmol/L.[13] Apolipoprotein(a) isoforms were measured as the total number of KIV repeats as previously described.[13] The analyses were based on size of the major apolipoprotein(a) KIV isoform visualized on agarose gel electrophoresis.[7] In this study, the major apolipoprotein(a) isoform was associated with the smaller of the 2 alleles in 87% of subjects.

Determination of Oxidized Phospholipids on Apolipoprotein B-100 Levels

Oxidized phospholipids on apolipoprotein B-100 (OxPL-apoB) were measured as previously described in detail by chemiluminescent ELISA using the murine monoclonal antibody E06, which binds to the phosphocholine (PC) headgroup of oxidized but not native phospholipids.[14-16] The OxPL-apoB values are expressed as relative lights units reflecting the amount of E06 bound to OxPL on apoB particles captured on microtiter well plates with antibody MB47. The OxPL-apoB measure primarily (~85-90%) reflects the OxPL on Lp(a), in which OxPL are bound both covalently to apo(a) and in the lipid phase of apoB, with the remaining (0-15%) OxPL being present on non-Lp(a) apoB particles.[17] It is to be emphasized that the OxPL-apoB measure only represents those OxPL recognized by E06 (i.e. E06 immunoreactivity) and does not represent non-E06 detectable OxPL present on apoB particles.[17]

Determination of Major Adverse Cardiovascular Events

The subjects were followed from January 2001 until December 31 2010 for a median of 9.5 years of follow-up. Event adjudication beyond 2010 is not currently available for the DHS. Major adverse cardiovascular events (MACE) was defined as cardiac death, non-fatal MI, stroke/TIA, unstable angina requiring hospitalization and arterial vascularization that included coronary artery bypass surgery, percutaneous coronary intervention, carotid endarterectomy, carotid stenting and peripheral artery revascularization. Of the 3419 individuals, all had complete *LPA* SNP, apolipoprotein(a) isoform and biomarker data. Additionally, all-cause death adjudicated until Dec 31 2010, however, this endpoint was not included in the MACE analyses as the causes of non-cardiac death were highly heterogeneous and would appropriately test the Lp(a) cardiovascular hypothesis. From this group of 3419 individuals, 2929 had the MACE endpoint adjudicated until the latest follow-up of Dec 31 2010, therefore the MACE analyses were performed on these subjects. There were no significant baseline differences in the individuals with or without adjudicated MACE events.

Statistical Analyses

Analyses were performed with SPSS 23.0 software package. Continuous variables were presented as means ± standard deviations (SD) or medians and interquartile range and dichotomous variables as percentages. Differences in baseline attributes between subjects were analyzed with ANOVA and χ^2-test. Correlations between variables were determined with the Spearman test. The base-2 logarithms ($\log_2$) of Lp(a), size of the major apolipoprotein(a) isoform, OxPL-apoB and triglycerides were used to account for skewness in the distributions. Thus, hazard ratios for these variables reflect the change in hazard for an increase of 1 $\log_2$ (the equivalent of a doubling of the value) in the measure. Multivariable Cox regression analysis was used to estimate the associations between *LPA* SNPs, the size of the major apolipoprotein(a) isoform, Lp(a) and OxPL-apoB levels and time to MACE, with adjustment for sex, age in deciles, smoking status, the presence or absence of hypertension and diabetes, body mass index and levels of LDL-C per 25 mg/dL increase, HDL-C per 10 mg/dL increase, and $\log_2$ triglycerides. If a patient had more than one MACE event, only the first event was counted.

Results

Baseline Characteristics of the Study Group by Ethnicity

Table 1 displays the baseline characteristics of the entire group and by individual ethnic group. Blacks tended to be older and have a higher prevalence of hypertension, diabetes and current smoking. Lp(a) and OxPL-apoB levels were highest in Blacks, followed by Whites, then Hispanics. A similar but inverse association was noted in the major apolipoprotein(a) isoform among ethnic groups. Blacks also had higher HDL-C and lower triglyceride levels compared to the other groups.

The prevalence of *LPA* SNPs is shown in Table 1 and all were significantly different among ethnic groups except G+1/inKIV-8A. Rs3798220 was most prevalent in Hispanics, rs10455872 in Whites, rs9457951 in Blacks, rs1081693 in Hispanics, rs41272110 in Whites.

All patients had adjudicated total mortality data, but the 490 patients without an adjudicated MACE event did not have differences in Lp(a) or OxPL-apoB than patients with adjudicated MACE (Supplemental Table 1). They tended to be younger, male, smokers, with more hypertension, diabetes, higher triglycerides, but lower BMI, total cholesterol and LDL-C.

Relationship of *LPA* SNPs to Lp(a), OxPL-apoB, and Apolipoprotein(a) Isoforms by Ethnicity

Table 2 displays the relationship of *LPA* SNPs to Lp(a), apolipoprotein(a) isoforms and OxPL-apoB.

For rs3798220, in Blacks, Lp(a) and OxPL-apoB levels were not significantly different among wild-type alleles, heterozygotes and homozygotes. In Whites, significant differences were noted in Lp(a), apolipoprotein(a) isoforms and OxPL-apoB ($p<0.001$ for all) with carriers of the C allele having highly elevated Lp(a) and OxPL-apoB levels and corresponding smaller isoforms. In Hispanics significant differences were noted in Lp(a), apolipoprotein(a) isoforms and OxPL-apoB ($p<0.001$ for all) but in contrast to Whites, an inverse association was present, with in carriers of the C allele having lower Lp(a), lower OxPL-apoB and larger isoforms sizes.

For rs10455872, Lp(a), OxPL-apoB, and apolipoprotein(a) isoforms were significantly different among wild-type alleles and heterozygotes and homozygotes among Blacks, Whites and Hispanics ($p<0.001$ in all 3 ethnic groups for each measure), with carriers of the G allele having highly elevated Lp(a) and OxPL-apoB levels and corresponding smaller isoforms.

For rs9457951, Lp(a), OxPL-apoB and apolipoprotein(a) isoforms were significantly different among wild-type alleles and heterozygotes and homozygotes among Blacks and Hispanics, but not Whites, with carriers of the G allele having highly elevated Lp(a) and OxPL-apoB levels. Blacks did not have corresponding smaller isoforms but Hispanics did.

For rs1801693, rs41272110, variable differences were noted Lp(a), OxPL-apoB and apolipoprotein(a) isoforms among ethnic groups and for G+1/inKIV-8A no differences were noted (Table 2).

Correlations Between *LPA* SNPs, Lp(a), OxPL-apoB and Size of the Major Apolipoprotein(a) Isoform

Table 3 displays the correlation between *LPA* SNPs and Lp(a), OxPL-apoB and size of the major apolipoprotein(a) isoform. rs3798220 was inversely associated with size of the major apolipoprotein(a) isoform in Whites, positively in Hispanics and not correlated in Blacks. rs10455872 was inversely associated with size of the major apolipoprotein(a) isoform in all 3 groups. rs9457951 was positively associated in Blacks, inversely in Hispanics and not correlated in Whites. Lp(a) was highly correlated with OxPL-apoB in Blacks (Spearman $r=0.87$, $p<0.001$), Whites ($r=0.70$, $p<0.001$) and Hispanics ($r=0.63$, $p<0.001$). The size of the major apolipoprotein(a) isoform was generally inversely associated with Lp(a) and OxPL-apoB. Finally, the 3 *LPA* SNPs did not correlate with each other in Blacks and Whites, but rs3798220 was negatively associated with rs10455872 in Hispanics.

For rs1801693, modest positive and inverse correlations were noted with Lp(a), OxPL-apoB, rs3798220 and rs10455872 depending on the ethnic group (Table 3).

Relationship of Lp(a), OxPL-apoB and Apolipoprotein(a) Isoforms to Time to MACE in All Subjects

Of the 2929 individuals with adjudicated MACE, there were 211 individuals (7.2% of total) with MACE events with 146 in Blacks, 52 in Whites and 13 in Hispanics over the median 9.5-year follow-up. A total of 336 (1.6 events per person with an event) events occurred, including 41 CV deaths, 61 MIs, 59 strokes, 19 TIAs, 25 cases of unstable angina, 65 PCIs, 33 CABGs, 12 carotid and 21 peripheral arterial revascularizations.

Table 4 shows the results of sequentially adjusted Cox regression analysis of Lp(a), OxPL-apoB and the major apolipoprotein(a) isoform with MACE in the entire group. Linear trends were significant for Lp(a), OxPL-apoB and apolipoprotein(a) isoforms across quartiles. In unadjusted analyses, the hazard ratios (HR) 95% confidence intervals (95% CI) quartiles 3 (1.87 (1.22-2.88), p=0.004) and 4 (2.44 (1.62-3.68), p<0.001) of Lp(a), quartile 4 (1.78 (1.22-2.59), p=0.003) of OxPL-apoB were significantly higher compared to quartile 1, and quartile 4 (0.60 (0.38-0.86), p=0.007) of the size of the major apolipoprotein(a) isoform had a lower HR compared to quartile 1. Adjustment for sex, HTN, diabetes, smoking, age in deciles, BMI, LDL per 25 mg/dL, HDL per 10 mg/dL and $\log_2$ triglycerides did not materially change the results for Lp(a), OxPL-apoB or size of the major apolipoprotein(a) isoform size. Addition of the 6 *LPA* SNPs to the model attenuated the risk slightly but significance was maintained for Lp(a), OxPL-apoB and size of the major apolipoprotein(a) isoform. For Lp(a), adding the size of the major apolipoprotein(a) isoform attenuated the risk further, and finally adding OxPL-apoB resulted in loss of significance. For OxPL-apoB and the size of the major apolipoprotein(a) isoform, similar findings were present with addition of Lp(a) fully attenuating risk. Removing arterial revascularization (PCI, CABG, carotid and peripheral arterial revascularization) for the MACE endpoint did not materially change the results.

Figure 1A-C displays the cumulative event-free survival curves for Lp(a) over the median 9.5-year follow-up, OxPL-apoB and size of the major apolipoprotein(a) isoform after adjustment for sex, HTN, DM, current smoking, BMI, age in deciles, LDL-C per increase of 25 mg/dl, $\log_2$ triglycerides, HDL-C per increase of 10 mg/dL, and by carrier status of rs3798220, rs15455872 and rs9457951, rs1801693, rs412722110 and G+1/inKIV-8A. Separation of MACE events was evident across all 4 quartiles.

Relationship of Lp(a), OxPL-apoB and Size of the Major Apolipoprotein (a) Isoform to Time to MACE by Ethnicity

In Blacks, adjustment for sex, HTN, diabetes, smoking, age in deciles, BMI, LDL per 25 mg/dL, HDL per 10 mg/dL and $\log_2$ triglycerides, Lp(a) was a positive predictor (HR 3.27 (1.27-7.11), p=0.012) and the size of the major apolipoprotein(a) isoform and inverse predictor (HR 0.49 (0.27-0.88), p=0.17) of time to MACE (Table 5). In Whites, the size of the major apolipoprotein(a) isoform was an inverse predictor (HR 0.49 (0.27-0.88), p=0.017), whereas strong trends were noted for Lp(a) and OxPL-apoB. In Hispanics, OxPL-

apoB (HR 13.4 (1.89-94,3), p=0.009) was a predictor of time to MACE, with Lp(a) a strong trend. The interaction tests between ethnicity and Lp(a), OxPL-apoB and the size of the major apolipoprotein(a) isoform were negative (p>0.05 for all).

Discussion

This study demonstrates that the prevalence and association of *LPA* SNPs with size of apolipoprotein(a) isoforms, Lp(a) and OxPL-apoB levels is highly variable and ethnicity-specific. Depending on their association with small or large isoforms, *LPA* SNPs may be associated with either higher or lower Lp(a) and OxPL-apoB levels in different ethnic groups. Importantly, irrespective of and independent of the SNP association, elevated plasma levels of Lp(a) or OxPL-apoB were independent predictors of MACE in the overall group and across ethnic groups. Smaller apolipoprotein(a) isoform sizes were predictive of incident MACE, but this relationship was abrogated by *LPA* SNPs or Lp(a)/OxPL-apoB levels. Finally, adding OxPL-apoB levels, which primarily reflects OxPL on Lp(a),[17,18] to the multivariable analysis abrogated the risk, consistent with the notion that much of the CVD risk of Lp(a) is driven by its content of pro-inflammatory OxPL.[16,19-26]

This study provides clarity the role of the *LPA* gene and elevated Lp(a) levels in predicting CVD risk by demonstrating that elevated circulating Lp(a) levels is the key variable in predicting CVD risk irrespective of ethnicity. Most of the prior data on *LPA* and Lp(a) have been generated in subjects of European descent. There has been significant controversy whether elevated Lp(a) levels are similarly associated with CVD risk in Blacks, who tend to have the highest average Lp(a) levels.[7,27] Interestingly, they tend to have larger isoforms that are associate with such levels, rather than small isoforms as has been shown in subjects of European descent.[28,29] Importantly, because they key *LPA* SNPs and apolipoprotein(a) isoform sizes were measured in this study, their relative contributions alone as well as in conjunction with Lp(a)-mediated risk were additionally determined.

Consistent with the role of Lp(a) and OxPL-apoB in predicting future MACE, it can be concluded that small isoforms are primarily important to CVD risk prediction in that they contribute to higher Lp(a)/OxPL-apoB levels via a shorter synthesis time in the hepatocyte.[30] Interestingly, this was noted seen in Whites and Hispanics and not Blacks, consistent with the fact that isoforms size explains <50% of circulating Lp(a) levels.[8]

These data further demonstrate that *LPA* SNPs are likely to be markers rather than mediators of elevated Lp(a) and/or OxPL-apoB and their association with Lp(a)/OxPL-apoB appears to be due to their random co-segregation with apolipoprotein(a) isoform sizes. Similarly, *LPA* SNPs appear to be tagging SNPs for isoform size but not necessarily small isoforms. For example, SNP rs3798220 was present in 42.76% of Hispanics, subjects, but only in 4.27% of Whites, yet was associated with large isoforms and lower Lp(a) levels in Hispanics but very small isoforms and higher Lp(a) levels in Whites. For clinical translatability and to address the clinical question on what is the optimal “Lp(a)” measure, these data suggest that circulating Lp(a) levels are the main variable in quantitating cardiovascular risk, rather than *LPA* SNPs or apolipoprotein(a) isoform size. Interestingly, in prior studies, OxPL-apoB was either a similar or a better reflector of Lp(a)-mediated risk, consistent with the fact that the

presence of OxPL on Lp(a) is variable and may reflect the flux of OxPL particles in different pathophysiological situations as well as the OxPL carrying capacity of Lp(a) as well as Lp(a) particle number.[22,23,25,31] For example, it was previously shown in DHS that high Lp(a) concentrations that are also associated small apo(a) isoforms (i.e. higher particle number) had the highest correlation with OxPL-apoB, irrespective of ethnic group status.[7]

Although the issue of ethnicity and Lp(a) risk has been controversial due to inconsistent results in individuals of African descent, this study adds to the growing data that ethnicity by itself is not the relevant variable, but the Lp(a) level within the ethnicity. Elevated Lp(a) in individuals of African descent, who have higher Lp(a), but also differences in the distribution of kringle repeats than Whites, is a risk factor as suggested by both the ARIC study with 20-yr follow-up,[27] as well as the recent results from the MESA study.[32]

Data in LPA SNPs is also highly variable according to ethnic status. For example, data derived primarily from European populations show that rs3798220 is a relatively infrequent SNP present in 2-4% of community subjects.[8,33-36] However, the frequency of this SNP is increased in subjects with elevated Lp(a) levels and 24% of Europeans who are in the >95th percentile for elevated Lp(a) (i.e. >139 mg/dL) carry this SNP.[36] rs3798220 is also more prevalent in individuals with cardiovascular disease, such as in individuals undergoing apheresis for elevated Lp(a) (>60 mg/dL) for recurrent cardiovascular events having an allele frequency of 26.2%.[37] Additionally, this SNP also associated with increasing levels of OxPL-apoB that are associated with increased cardiovascular risk.[38,39] In European populations this SNP is associated with small apolipoprotein(a) isoforms, usually <22 KIV repeats, and markedly elevated Lp(a) levels, particularly in homozygotes. Interestingly, this SNP is not present at all in Africans,[40] is present in <10% in Asian Indians but is more prevalent (11.6%) in East Asians such as Chinese in Hong Kong.[41,42] *LPA* SNP rs10455872 is present in 7-15% of the general population in Europeans.[8,43,44] Its prevalence increases with increasing Lp(a) levels and is present in 47% of northern Europeans with Lp(a) levels >95th percentile. rs10455872 was prevalent in 37.2% of individuals undergoing apheresis with progressive coronary artery disease.[37] Less is known about this SNP in non-European populations, but it was not associated with cardiovascular disease or small isoforms in Japan in a genome wide association study or other similar East Asian populations.[42] The prevalence of rs9457951 is primarily associated with Black subjects but it only explains approximately 5% of the contribution to Lp(a) levels.[9]

Limitations of this study include the fact that the Hispanic group was smallest in size and had few events, therefore the role of the *LPA* gene, Lp(a) and OxPL-apoB levels may be underpowered for the MACE endpoint.

In conclusion, the relationship of the *LPA* gene to MACE is best explained by elevated plasma Lp(a) or OxPL-apoB levels and not by *LPA* SNPs or size of apolipoprotein(a) isoforms. Elevated Lp(a) levels, irrespective of ethnicity, are a predictor of future MACE. Studies with potent and specific Lp(a) lowering drugs are underway to test the hypothesis that lowering Lp(a) will reduce CVD risk in subjects with elevated Lp(a).[12]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding Sources: NIH R01-HL119828, P01-HL088093, P01-HL055798, R01-HL093767 R01-HL086599 (ST, JLW).

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Clinical Perspective

1. What is new?

- The prevalence of LPA snps and apolipoprotein(a) isoforms are very different across ethnic groups.
- LPA snps that are associated with elevated Lp(a) in Whites are associated with low Lp(a) in Hispanics, mainly due to differences in apolipoprotein(a) isoforms size.
- After multivariable adjustment Lp(a) and OxPL-apoB are both predictors of MACE in a multi-ethnic cohort.
- LPA snps and apolipoprotein(a) isoforms do not add predictive value to models when Lp(a) or OxPL-apoB is included.

2. What are the clinical implications?

- Elevated Lp(a) and OxPL-apoB are predictors of MACE across racial groups.
- The data suggest that much of Lp(a)-mediated MACE is driven by OxPL.
- LPA snps and isoforms did not have clinical utility in this study and further research is needed to assess whether they add additional clinical value in specific patient populations.

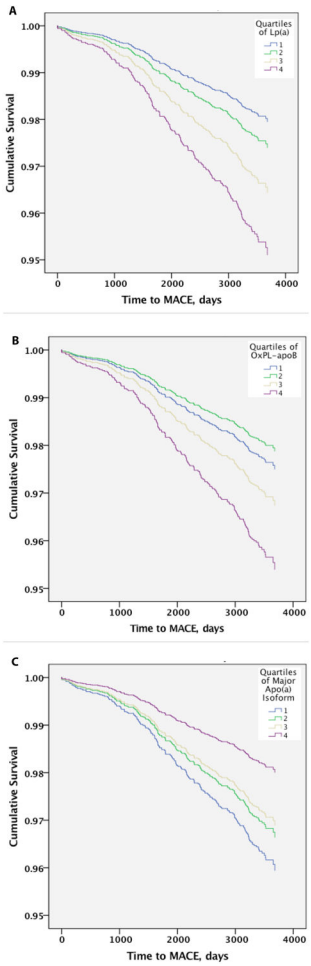

Figure 1.

Relationship of Lp(a) (A), OxPL-apoB (B) and size of the major apolipoprotein(a) isoform (C) to time to MACE by multivariable adjusted Cox regression analysis.

Table 1

Baseline characteristics of the study groups as a whole and by ethnicity.

	Entire Group (N=3419)	Black (N=1792)	White (N=1030)	Hispanic (N=597)	P-Value for ethnicity
Age, years (SD)	43.8 (10.1)	44.5 (10.2)	44.7 (10.0)	40.0 (9.2)	<0.001
Male, N (%)	1501 (43.9)	755 (42.1)	496 (48.2)	250 (42.0)	0.004
BMI	30.8 (7.6)	31.6 (8.2)	29.0 (6.7)	30.3 (6.6)	<0.001
HTN, N (%)	963 (28.2)	660 (36.8)	218 (21.2)	85 (14.2)	<0.001
Diabetes, N (%)	393 (11.5)	254 (14.2)	66 (6.4)	71 (11.9)	<0.001
Current smoking, N (%)	1004 (29.4)	598 (33.4)	284 (27.6)	122 (20.4)	<0.001
Laboratory variables					
Lp(a), nmol/L	49.9 (19.6-110.5)	79.0 (43-132)	26.9 (10-69)	21.3 (9-46)	<0.001
OxPL-apoB, RLU	4025 (2564-8734)	6445 (3419-11389)	3014 (2233-5306)	2689 (2058-3766)	<0.001
Size of major isoform, # kringles	24 (19.5-28)	23.0 (20-26)	24.0 (19-29)	27.0 (21-32)	<0.001
Total Cholesterol, mg/dl	180.3 (39.7)	177.7 (40.3)	183.6 (38.3)	182.3 (40.1)	<0.001
LDL-C, mg/dl	106.2 (35.5)	104.7 (36.8)	108.2 (34.3)	107.2 (33.3)	0.029
HDL-C, mg/dl	49.9 (14.9)	52.3 (15.4)	48.4 (15.1)	45.7 (11.2)	<0.001
VLDL-C, mg/dl	24.2 (18.9)	20.8 (17.7)	26.9 (18.1)	29.4 (21.5)	<0.001
Triglycerides, mg/dl	96.0 (67-146)	85.0 (62-123)	109.0 (75-166)	119.0 (81-176)	<0.001
Minor allele frequency, N (%)					
rs3798220	324 (9.48)	27 (1.51)	44 (4.27)	253 (42.38)	<0.001
rs10455872	213 (6.22)	33 (1.84)	147 (14.27)	33 (5.53)	<0.001
rs9457951	615 (17.99)	590 (32.92)	4 (0.0039)	21 (3.52)	<0.001
rs1801693	1341 (40.2)	417 (23.9)	532 (52.9)	392 (67.1)	<0.001
rs41272110	455 (13.4)	80 (4.48)	272 (26.72)	103 (17.37)	<0.001
G+1/inKIV-8A	186 (5.5)	102 (5.74)	55 (5.40)	29 (4.94)	0.75

Values are given as mean (SD) or median (IQR). For rs1801693, rs41272110 and G+1/inKIV-8A 3379, 3333 and 3395 subjects were genotyped.

Table 2

Relationship of *LPA* rs3798220, rs10485774 and rs9459571 to Lp(a), apolipoprotein(a) isoforms and OxPL-apoB.

	Black			
rs3798220	**TT (N = 1765)**	**TC (N= 27)**	**CC (N = 0)**	**P-Value**
Lp(a), nmol/L	79.3 (43.8-132.2)	49.1 (13.5-122.6)	-	0.15
OxPL-apoB, RLU	6507 (3510-11416)	4305 (2501-8733)	-	0.11
Major apolipoprotein(a) isoform, #KIV repeats	23.0 (20-26)	23.0 (21-33)	-	0.042
	White			
	TT (N = 985)	TC (N= 43)	CC (N = 1)	
Lp(a), nmol/L	25.9 (9.5-58.1)	187.3 (113.4-229.1)	197.1 (-)	<0.001
OxPL-apoB, RLU	2963 (2217-4701)	12493 (8521-16202)	13222 (-)	<0.001
Major apolipoprotein(a) isoform, #KIV repeats	24.5 (19-29)	17.0 (17-19)	17.0 (-)	<0.001
	Hispanic			
	TT (N = 343)	TC (N= 212)	CC (N = 41)	
Lp(a), nmol/L	25.2 (10.2-61.0)	18.8 (8.1-36.6)	13.2 (6.4-22.9)	<0.001
OxPL-apoB, RLU	2873 (2145-4567)	2586 (1972-3360)	2445 (1777-2901)	<0.001
Major apolipoprotein(a) isoform, #KIV repeats	25.0 (20-30)	30.25 (24-34)	33.0 (31.5-34)	<0.001
	Black			
rs10485774	AA (N = 1759)	AG (N= 33)	GG (N = 0)	P-Value
Lp(a), nmol/L	77.9 (43.4-132.1)	207.0 (117-261.9)	-	<0.001
OxPL-apoB, RLU	6314 (3473-11300)	11854 (6397-17005)	-	<0.001
Major apolipoprotein(a) isoform, #KIV repeats	23.0 (20-26)	17 (16-17.5)	-	<0.001
	White			
	AA (N = 882)	AG (N= 139)	GG (N = 8)	
Lp(a), nmol/L	21.2 (8.1-41.8)	141.4 (108.4-172.3)	219.6 (205.6-276.9)	<0.001
OxPL-apoB, RLU	2826 (2134-3895)	7949 (5496-10745)	12534 (8792-16924)	<0.001
Major apolipoprotein(a) isoform, #KIV repeats	25.5 (21-29)	17.0 (16-18)	17.25 (16.1-17.5)	<0.001
	Hispanic			
	AA (N = 563)	AG (N= 31)	GG (N = 2)	
Lp(a), nmol/L	19.6 (8.1-40.7)	151.7 (102.6-198.2)	151.3 (-)	<0.001
OxPL-apoB, RLU	2602 (2046-3530)	7966 (4773-12056)	8583 (-)	<0.001
Major apolipoprotein(a) isoform, #KIV repeats	28.0 (22-32)	17.0 (16-17)	17.0 (-)	<0.001
		Black		
rs9459571	CC (N = 1202)	CG (N= 547)	GG (N = 43)	P-Value
Lp(a), nmol/L	73.8 (40.6-121.9)	94.4 (50.8-153.4)	97.0 (62.2-186.6)	<0.001

	Black			
rs3798220	**TT (N = 1765)**	**TC (N= 27)**	**CC (N = 0)**	**P-Value**
OxPL-apoB, RLU	5502 (3247-9864)	8602 (4241-14223)	8032 (5052-17154)	<0.001
Major apolipoprotein(a) isoform, #KIV repeats	22.5 (20-26)	24.0 (20-27)	24.0 (19-24)	0.008
		White		
	CC (N = 1025)	CG (N= 3)	GG (N = 1)	
Lp(a), nmol/L	26.7 (10.1-68.7)	89.8 (-)	35.8 (-)	0.38
OxPL-apoB, RLU	3015 (2233-5286)	8223 (-)	1952 (-)	0.43
Major apolipoprotein(a) isoform, #KIV repeats	24.0 (19-29)	26.0 (-)	27.0 (-)	0.85
		Hispanic		
	CC (N = 575)	CG (N= 20)	GG (N = 1)	
Lp(a), nmol/L	20.6 (8.2-43.7)	76.4 (32.6-146.6)	507 (-)	<0.001
OxPL-apoB, RLU	2656 (2053-3674)	6508 (2993-11918)	18675 (-)	<0.001
Major apolipoprotein(a) isoform, #KIV repeats	28.0 (21-32)	21.5 (6508)	14 (-)	0.003
		Black		
rs1801693	AA (N = 1326)	AG (N= 391)	GG (N = 26)	P-Value
Lp(a), nmol/L	84.3 (49.5-134.7)	55.7 (30.6-116.5)	44.2 (12.0-98.0)	<0.001
OxPL-apoB, RLU	7066 (3884-11613)	4482 (2893-9923)	3836 (2459-9549)	<0.001
Major apolipoprotein(a) isoform, #KIV repeats	23.0 (20-26)	24.0 (20-28)	25.0 (19.75-32)	<0.001
		White		
	AA (N = 474)	AG (N= 434)	GG (N = 98)	
Lp(a), nmol/L	28.5 (11.7-80.1)	23.8 (8.6-57.1)	24.9 (4.2-108.2)	0.025
OxPL-apoB, RLU	3104 (2250-5773)	2958 (2229-4635)	3127 (2134-8486)	0.29
Major apolipoprotein(a) isoform, #KIV repeats	23.25 (18-29)	25.0 20-28	24.25 (18-29)	0.068
		Hispanic		
	AA (N = 192)	AG (N= 266)	GG (N = 126)	
Lp(a), nmol/L	29.5 (13.4-78.5)	19.6 (6.8-40.6)	16.4 (7.8-36.8)	<0.001
OxPL-apoB, RLU	2898 (2172-3587)	2739 (2049-3587)	2520 (1850-3570)	0.016
Major apolipoprotein(a) isoform, #KIV repeats	24.0 (19-28)	28.0 (23-33)	31.0 (25.38-33.63)	<0.001
		Black		
rs41272110	TT (N = 1704)	TG (N= 78)	GG(N = 2)	P-Value
Lp(a), nmol/L	79.4 (43.9-132.8)	59.3 (31.4-124.8)	60.5 (-)	0.33
OxPL-apoB, RLU	6509 (3521-11428)	4769 (2960-10707)	6317 (-1)	0.37
Major apolipoprotein(a) isoform, KIV repeats	23.0 (20-26.5)	21.5 (18-25)	22.5 (-)	0.071
		White		
	TT (N = 746)	TG (N= 258)	GG(N = 14)	
Lp(a), nmol/L	26.4 (7.3-76.6)	27.2 (13.7-58.4)	24.9 (13.4-106.7)	0.51
OxPL-apoB, RLU	3097 (265-5576)	2920 (2096-5036)	2575 (2170-4421)	<0.001

	Black			
rs3798220	**TT (N = 1765)**	**TC (N= 27)**	**CC (N = 0)**	**P-Value**
Major apolipoprotein(a) isoform, #KIV repeats	26.0 (19-29.5)	20.25 (18-25.63)	19.0 (18-20)	0.56
		Hispanic		
	TT (N = 490)	TG (N= 99)	GG(N = 4)	
Lp(a), nmol/L	20.6 (7.1-47.7)	23.5 (14.1-33.9)	21.4 (5.4-35.0)	0.47
OxPL-apoB, RLU	2774 (2056-3962)	2519 (2050-3468)	2566 (2264-3117)	0.62
Major apolipoprotein(a) isoform, #KIV repeats	28.5 (23-33)	20.0 (19-26)	19.5(19-22)	<0.001
		Black		
G+1/inKIV-8A	GG(N = 1673)	GA N(= 98)	AA(N = 4)	P-Value
Lp(a), nmol/L	78.9 (43.5-131.5)	77.6(50.8-153.4)	70.4 (30.8-91.2)	0.90
OxPL-apoB, RLU	6481 (3520-11305)	5845 (3036-13624)	5956 (2972-14026)	0.99
Major apolipoprotein(a) isoform, #KIV repeats	23.0 (20-26)	22.75 (20-26)	25.5 (19.5-31.5)	0.60
		White		
	GG (N = 962)	GA(N= 52)	AA(N = 3)	
Lp(a), nmol/L	26.4 (10.1-68.9)	32.3 (9.0-92.2)	37.5 ()	0.66
OxPL-apoB, RLU	3020 (2239-5296)	2984 (2262-5648)	1812 (-)	0.63
Major apolipoprotein(a) isoform, #KIV repeats	24.0 (19-29)	23.5 (17.25-27.75)	19.0 (-)	0.30
		Hispanic		
	GG (N = 558)	GA (N= 28)	AA(N = 1)	
Lp(a), nmol/L	21.1 (8.7-45.4)	24.8 (7.5-70.3)	5.1 (-)	0.72
OxPL-apoB, RLU	2720 (2070-3730)	2593 (2134-4741)	1708 (-)	0.71
Major apolipoprotein(a) isoform, #KIV repeats	27.0 (21-32)	28.0 (19.5-31.0)	34.0 (-)	0.43

Values are given as median (IQR).

Table 3

Spearman correlations of *LPA* single nucleotide polymorphisms with Lp(a), OxPL-apoB and size of the major apolipoprotein(a) isoform

	rs10455872	rs9457951	rs1801693	rs412722110	G+1/inKIV-8A	Lp(a)	OxPL-apoB	Major apolipoprotein(a) Isoform
Black								
rs3798220	-0.02	-0.07	0.22†	-0.005	-0.03	-0.05*	-0.05*	0.03
rs10455872	-	-0.07	-0.06*	-0.03	0.02	-0.16†	0.09†	-0.18†
rs9457951		-	-0.08	-0.07*	0.002	0.15†	0.21†	0.09†
rs1801693			-	-0.04	-0.005	0.17†	-0.15†	0.09
rs412722110				-	-0.02	-0.05*	-0.04	-0.06*
G+1/inKIV-8A					-	-0.006	-0.01	-0.002
Lp(a)						-	0.87†	-0.62†
OxPL-apoB								-0.48†
White								
rs3798220	-0.04	-0.01	0.23†	-0.05	0.05	0.22†	0.24†	-0.18†
rs10455872	-	-0.03	-0.18†	-0.11†	0.02	0.48†	0.42†	-0.50†
rs9457951		-	-0.04	0.04	0.05	0.04	0.02	0.01
rs1801693			-	-0.003	-0.04	0.08†	-0.02	0.07
rs412722110				-	0.003	-0.05	-0.04	-0.22†
G+1/inKIV-8A					-	-0.02	-0.02	-0.04
Lp(a)						-	0.70†	-0.56†
OxPL-apoB							-	-0.47†
Hispanic								
rs3798220	-0.18†	-0.08	0.64†	-0.10*	0.06	0.17†	-0.16†	0.37†
rs10455872	-	-0.05	-0.17†	-0.09*	-0.02	0.33†	0.30†	-0.37†
rs9457951		-	-0.14†	-0.04	-0.002	0.18†	0.16†	-0.13†
rs1801693			-	-0.16†	0.02	-0.17†	-0.12†	0.34†
rs412722110				-	0.002	0.03	-0.05	-0.31†
G+1/inKIV-8A					-	0.007	-0.003	-0.01

	rs10455872	rs9457951	rs1801693	rs412722110	G+1/inKIV-8A	Lp(a)	OxPL-apoB	Major apolipoprotein(a) Isoform
Lp(a)						-	0.63 †	-0.57 †
OxPL-apoB								-0.43 †

* p<-05

† p<0.001

Table 4

Hazard Ratios for MACE According to Quartiles of Lp(a), OxPL-apoB and Size of the Major Apolipoprotein(a) Isoform with Sequential adjustment.

Variable	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P-Value Q1 vs. Q4
Lp(a)	1.00	1.21 (0.76-1.92)	1.87 (1.22-2.88)	2.44 (1.62-3.68)	<0.001
Plus sex, HTN, diabetes, smoking, age in deciles, BMI, LDL-C per 25 mg/dL, HDL-C per 10 mg/dL, $\log_2$ TG	1.00	1.30 (0.80-2.12)	1.80 (1.13-2.87)	2.35 (1.50-3.69)	<0.001
Plus *LPA* SNPs	1.00	1.28 (0.76-2.13)	1.75 (1.07-2.88)	2.43 (1.48-3.97)	<0.001
Plus $\log_2$ apolipoprotein(a) major isoform	1.00	1.23 (0.73-2.06)	1.59 (0.94-2.68)	2.06 (1.17-3.62)	0.012
Plus $\log_2$ OxPL-apoB	1.00	1.23 (0.73-2.08)	1.46 (0.79-2.69)	1.69 (0.75-3.82)	0.21
OxPL-apoB	1.00	0.79 (0.50-1.24)	1.37 (0.92-2.03)	1.78 (1.22-2.59)	0.003
Plus sex, HTN, diabetes, smoking, age in deciles, BMI, LDL-C per 25 mg/dL, HDL-C per 10 mg/dL, $\log_2$ TG	1.00	0.86 (0.54-1.36)	1.32 (0.87-2.00)	1.89 (1.26-2.84)	0.003
Plus *LPA* SNPs	1.00	0.85 (0.52-1.38)	1.31 (0.84-2.04)	1.80 (1.21-2.87)	0.005
Plus $\log_2$ apolipoprotein(a) major isoforms	1.00	0.84 (0.51-1.36)	1.17 (0.74-1.84)	1.58 (0.99-2.51)	0.056
Plus $\log_2$ Lp(a)	1.00	0.69 (0.42-1.15)	0.74 (0.46-1.36)	0.90 (0.48-1.69)	0.73
Major apolipoprotein(a) isoform size	1.00	1.03 (0.72-1.47)	0.97 (0.67-1.40)	0.60 (0.38-0.86)	0.007
Plus sex, HTN, diabetes, smoking, age in deciles, BMI, LDL-C per 25 mg/dL, HDL-C per 10 mg/dL, $\log_2$ TG	1.00	0.79 (0.55-1.14)	0.77 (0.53-1.13)	0.52 (0.34-0.80)	0.003
Plus *LPA* SNPs	1.00	0.83 (0.55-1.24)	0.76 (0.50-1.17)	0.49 (0.30-0.78)	0.003
Plus $\log_2$ OxPL-apoB	1.00	0.84 (0.56-1.27)	0.94 (0.59-1.45)	0.62 (0.37-1.05)	0.076
Plus $\log_2$ Lp(a)	1.00	0.99 (0.64-1.52)	1.05 (0.66-1.66)	0.79 (0.65-1.37)	0.40

Quartiles for Lp(a): Q1<19.6, Q2 >19.6 - <49.9, Q3 >49.9 - <110.5, Q4 >110.5 nmol/L. Quartiles for major isoform size: Q1<19.5, Q2 >19.5 - <24.0, Q3 >24.0 - <28.0, Q4 >28.0 Kringle IV repeats. Quartiles for OxPL-apoB: Q1<2564, Q2 >2564 - <4025, Q3 >4025 - <8734, Q4 >8734 RLU.

Table 5

Hazard Ratios for MACE by Ethnicity According to Quartiles of Lp(a), OxPL-apoB and Size of the Major Apolipoprotein(a) Isoform with Sequential adjustment.

	Black				
Variable	**Quartile 1**	**Quartile 2**	**Quartile 3**	**Quartile 4**	**P-Value Q1 vs. Q4**
Lp(a) plus sex, HTN, diabetes, smoking, age in deciles, BMI, LDL-C per 25 mg/dL, HDL-C per 10 mg/dL, $\log_2$ TG	1.00	1.80 (0.73-4.43)	2.37 (0.99-5.63)	3.27 (1.27-7.11)	0.012
OxPL-apoB plus sex, HTN, diabetes, smoking, age in deciles, BMI, LDL-C per 25 mg/dL, HDL-C per 10 mg/dL, $\log_2$ TG	1.00	0.86 (0.44-1.68)	1.13 (0.63-2.02)	1.54 (0.87-2.72)	0.140
Major apolipoprotein(a) isoform size plus sex, HTN, diabetes, smoking, age in deciles, BMI, LDL-C per 25 mg/dL, HDL-C per 10 mg/dL, $\log_2$ TG	1.00	0.81 (0.52-1.25)	0.76 (0.48-1.20)	0.49 (0.27-0.88)	0.017
	White				
Variable	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P-Value Q1 vs. Q4
Lp(a) plus sex, HTN, diabetes, smoking, age in deciles, BMI, LDL-C per 25 mg/dL, HDL-C per 10 mg/dL, $\log_2$ TG	1.00	0.88 (0.42-1.88)	1.31 (0.56-3.03)	1.83 (0.88-3.80)	0.105
OxPL-apoB plus sex, HTN, diabetes, smoking, age in deciles, BMI, LDL-C per 25 mg/dL, HDL-C per 10 mg/dL, $\log_2$ TG	1.00	0.54 (0.24-1.23)	0.75 (0.34-1.63)	0.57 (0.28-1.17)	0.127
Major apolipoprotein(a) isoform size plus sex, HTN, diabetes, smoking, age in deciles, BMI, LDL-C per 25 mg/dL, HDL-C per 10 mg/dL, $\log_2$ TG	1.00	0.81 (0.52-1.25)	0.76 (0.48-1.20)	0.49 (0.27-0.88)	0.017
	Hispanic				
Variable	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P-Value Q1 vs. Q4
Lp(a) plus sex, HTN, diabetes, smoking, age in deciles, BMI, LDL-C per 25 mg/dL, HDL-C per 10 mg/dL, $\log_2$ TG	1.00	3.44 (0.55-21.7)	2.29 (0.38-13.8)	5.43 (0.79-37.2)	0.085
OxPL-apoB plus sex, HTN, diabetes, smoking, age in deciles, BMI, LDL-C per 25 mg/dL, HDL-C per 10 mg/dL, $\log_2$ TG	1.00	1.66 (0.31-8.82)	1.07 (0.10-11.2)	13.4 (1.89-94.3)	0.009
Major apolipoprotein(a) isoform size plus sex, HTN, diabetes, smoking, age in deciles, BMI, LDL-C per 25 mg/dL, HDL-C per 10 mg/dL, $\log_2$ TG	1.00	0.38 (0.04-3.80)	0.68 (0.11-4.03)	0.35 (0.06-1.91)	0.23

Quartiles for Lp(a): Q1<19.6, Q2 >19.6 - <49.9, Q3 >49.9 - <110.5, Q4 >110.5 nmol/L. Quartiles for major isoform size: Q1<19.5, Q2 >19.5 - <24.0, Q3 >24.0 - <28.0, Q4 >28.0 Kringle IV repeats. Quartiles for OxPL-apoB: Q1<2564, Q2 >2564 - <4025, Q3 >4025 - <8734, Q4 >8734 RLU