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Apolipoprotein(a) Genetic Sequence Variants Associated With Systemic Atherosclerosis and Coronary Atherosclerotic Burden But Not With Venous Thromboembolism

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Objectives The purpose of this study is investigate the effects of variants in the apolipoprotein(a) gene (*LPA*) on vascular diseases with different atherosclerotic and thrombotic components.

Background It is unclear whether the *LPA* variants rs10455872 and rs3798220, which correlate with lipoprotein(a) levels and coronary artery disease (CAD), confer susceptibility predominantly via atherosclerosis or thrombosis.

Methods The 2 *LPA* variants were combined and examined as *LPA* scores for the association with ischemic stroke (and TOAST [Trial of Org 10172 in Acute Stroke Treatment] subtypes) (effective sample size [n_e] = 9,396); peripheral arterial disease (n_e = 5,215); abdominal aortic aneurysm (n_e = 4,572); venous thromboembolism (n_e = 4,607); intracranial aneurysm (n_e = 1,328); CAD (n_e = 12,716), carotid intima-media thickness (n = 3,714), and angiographic CAD severity (n = 5,588).

Results *LPA* score was associated with ischemic stroke subtype large artery atherosclerosis (odds ratio [OR]: 1.27; $p = 6.7 \times 10^{-4}$), peripheral artery disease (OR: 1.47; $p = 2.9 \times 10^{-14}$), and abdominal aortic aneurysm (OR: 1.23; $p = 6.0 \times 10^{-5}$), but not with the ischemic stroke subtypes cardioembolism (OR: 1.03; p = 0.69) or small vessel disease (OR: 1.06; p = 0.52). Although the *LPA* variants were not associated with carotid intima-media thickness, they were associated with the number of obstructed coronary vessels ($p = 4.8 \times 10^{-12}$). Furthermore, CAD cases carrying *LPA* risk variants had increased susceptibility to atherosclerotic manifestations outside of the coronary tree (OR: 1.26; p = 0.0010) and had earlier onset of CAD (–1.58 years/allele; $p = 8.2 \times 10^{-8}$) than CAD cases not carrying the risk variants. There was no association of *LPA* score with venous thromboembolism (OR: 0.97; p = 0.63) or intracranial aneurysm (OR: 0.85; p = 0.15).

Conclusions *LPA* sequence variants were associated with atherosclerotic burden, but not with primarily thrombotic phenotypes. (J Am Coll Cardiol 2012;60:722–9) © 2012 by the American College of Cardiology Foundation

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Recent studies strongly support a causal relationship between circulating lipoprotein (a) (Lp[a]) and coronary artery disease (CAD) (1–3). However, the pathophysiologic mechanism of this relationship is unclear. Substantial experimental evidence supports the notion that Lp(a) has both atherogenic and thrombogenic properties (4–7), suggesting that Lp(a) may have a role in both atherosclerotic and thrombotic aspects of arterial disease and potentially also in venous thrombosis.

Alleles of 2 single nucleotide polymorphisms (SNPs) in the *LPA* gene, *rs10455872* and *rs3798220,* have been shown to be associated with high plasma levels of Lp(a) and CAD (1,8). The variants, which are poorly correlated ($r^2 < 0.001$), together explain about 36% of the variance in Lp(a) levels (1). Each minor allele of *rs10455872* (G; 6.2% frequency) and *rs3798220* (C; 1.4% frequency) increased log Lp(a) by 1.08 and 1.15 standard deviation units and the risk for CAD by 47% and 68%, respectively (1). Evidence for the association between plasma Lp(a) and other atherosclerotic and thrombotic diseases is weaker; some studies have supported such an association, but others have not (2,3,9–18). Furthermore, it has not been established whether Lp(a), or Lp(a) correlated variants, confer the same risks to all of the ischemic stroke TOAST (Trial of Org 10172 in Acute Stroke Treatment) subtypes (19,20). Therefore, additional information about the role of Lp(a) at different vascular sites obtained from relatively large datasets that supports or refutes previously found associations is needed.

The objective of this study was to investigate the effects of the *LPA* variants on the risks for vascular diseases with different atherosclerotic and thrombotic components, to provide insight into the mechanism by which Lp(a) confers susceptibility. We investigated the association of the aforementioned Lp(a) correlated SNPs, with vascular diseases related to atherosclerosis and/or thrombosis: ischemic stroke (and its TOAST subtypes), peripheral arterial disease (PAD), abdominal aortic aneurysm (AAA), venous thromboembolism (VTE), and CAD in many case-control series. We also tested for association with intracranial aneurysm (IA), an arterial disease without arteriosclerotic or thrombotic etiology. Additionally, the effects of *LPA* SNPs on atherosclerotic burden, as well as on carotid intima-media thickness (IMT), an early marker of atherosclerosis, were examined.

Methods

Study populations. Samples from 35 case-control series that included patients with ischemic stroke (effective sample size [n_e] = 9,396), PAD (n_e = 5,215), AAA (n_e = 4,572), VTE (n_e = 4,607), IA (n_e = 1,328), and CAD (n_e = 12,716), as well as from 3,714 subjects with carotid IMT measurements, were analyzed. Samples from 2 cross-sectional studies, including 5,588 subjects who had undergone coronary angiography, were used to assess the association with CAD severity, as well as the association with myocardial infarction (MI), among those with angiographic CAD. The association with age at onset of CAD was assessed in 9,608 cases. See further description in the Online Appendix.

Ethical approval. All studies involved in the current analyses were approved by local research ethics committees and informed consent was obtained from all participants.

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Genotyping. DNA extraction and single SNP genotyping was carried out with various high-throughput methods with laboratory-specific quality-control procedures as detailed in Online Table 1. As the *LPA* SNPs were not represented on available genome-wide chips, genotypes for the Icelandic samples were imputed using methods previously described (21).

Statistical analysis. For the main analyses, we assessed the association between the outcome variables and the total number of minor alleles of either SNP (*rs3798220[C]* or *rs10455872[G]*), assuming that both minor alleles have the same effect on the outcome. The effect of each SNP on the binary outcomes, as reported in Online Table 2, was also assessed by comparing it with the effect of the major alleles of both SNPs.

Logistic and linear regression models were used for binary and quantitative outcomes, respectively, to test for linear trend with allele score (*LPA* score defined as the number of minor alleles of *rs3798220* and *rs10455872*) to derive effect estimates, standard errors, and 2-sided p values, assuming additive allelic effects (on the linear or log scale as appropriate). These tests were performed as implemented in NEMO (22), the R-software, and STATA software version 10.

The IMT trait was logarithmically transformed before analysis, and adjusted for age, sex, body mass index, physical exercise, and study center (country of origin). Age at angiography and sex were included as covariates in the linear regression model when fitting the number of affected coronary vessels to the *LPA* score. In the combined analysis, additional adjustments were made for study site and ethnicity.

To allow for the relatedness between subjects in the GAIT (Genetic Analysis of Idiopathic Thrombophilia) studies, the method of generalized estimating equations was used as implemented in the generalized estimating equations package (23) for the R-software. For the Icelandic studies, p values and 95% confidence interval (CI) of ORs are given after adjustment for the relatedness of the subjects and other possible population stratification using the method of genomic control. As the association tests for IMT in the Oxfordshire Family Blood Pressure studies were not significant, no adjustments for relatedness were made.

A fixed-effects meta-analysis approach was used to estimate an inverse-variance weighted average of the ORs from all studies of each outcome category. Tests of whether summary ORs were equal to 1, and whether the true effects in all studies were the same (likelihood ratio chi-square heterogeneity test) were performed. Allelic frequencies of *rs3798220(C)* and *rs10455872(G),* provided in the introduction, are the average frequency of the 15 populations of European origin examined in this study.

Given the number of traits tested, we considered p values <0.0017 significant, assuming a Bonferroni correction factor of 30. The power of the study to detect significant association ($p \leq 0.0017$, assuming an OR of 1.3) between *LPA* score and ischemic stroke, PAD, AAA, or VTE, was >97%. The power was slightly less for the ischemic stroke subtypes large artery atherosclerosis (LAA), cardioembolism (CE), or small vessel disease (SVD), or 74%, 78%, and 52%, respectively, and for IA the power was 34%. The power to detect an association between *LPA* score and IMT with effect size ≥0.1 mm was >99%, at a p value even as low as 0.0005. Prospective studies have suggested that a 0.1-mm increase in IMT is associated with a 20% to 30% increase in risk for developing CAD (24,25), and each *LPA* minor allele increases the risk for CAD by 20% to 30%. An effect size of 0.1 mm per variant allele could therefore be expected if the effect of *LPA* variants on CAD (or other atherosclerotic diseases) was mediated entirely through early atherogenesis and reflected in changes in the carotid artery IMT.

As the case-control ratios differed between studies, the effective sample size (n_e), representing the sample size for cases in which the case-control ratio was 1, for each study was calculated as the harmonic mean of the number of cases and controls and is reported in Figures 1 and 2. For the Icelandic studies, the effect sample size was further adjusted for relatedness by dividing it by the genomic control inflation factor. The combined effective sample sizes were used in the power calculations.

Abbreviations and Acronyms

AAA = abdominal aortic aneurysm
CAD = coronary artery disease
CE = cardioembolism
CI = confidence interval
IA = intracranial aneurysm
IMT = intima-media thickness
LAA = large artery atherosclerosis
Lp(a) = apolipoprotein (a)
LPA = apolipoprotein(a) gene
MI = myocardial infarction
n_e = effective sample size
OR = odds ratio
PAD = peripheral arterial disease
SNP = single nucleotide polymorphism
SVD = small vessel disease
TOAST = Trial of Org 10172 in Acute Stroke Treatment
VTE = venous thromboembolism

Results

***LPA* SNP association with different vascular traits.** The associations between each SNP and the vascular diseases are shown for all study groups in Online Tables 2A to 2D. As the estimated effects of the 2 SNPs were not significantly different, and the mechanisms by which both SNPs affect Lp(a) have been suggested to be similar (1), for reasons of simplicity (and to increase power) the main analyses (Figs. 1 and 2, Tables 1, 2, and 3) report the effect estimates for the 2 SNPs combined (*LPA* score), which assumes that their effects are equal. *LPA* score results for each study group are detailed in Online Tables 3A to 3C.

Results from all studies combined show nominally significant association between the *LPA* score and ischemic stroke overall, with an estimated OR of 1.10 (95% CI: 1.02 to 1.18; $p = 0.016$) (Fig. 1). Ischemic stroke subtype analysis revealed that the association is restricted to LAA (OR: 1.27; 95% CI: 1.11 to 1.46; $p = 6.7 \times 10^{-4}$), as the effects of *LPA* variants on the CE and SVD subtypes were nonsignificant ($p > 0.50$). The *LPA* score was associated

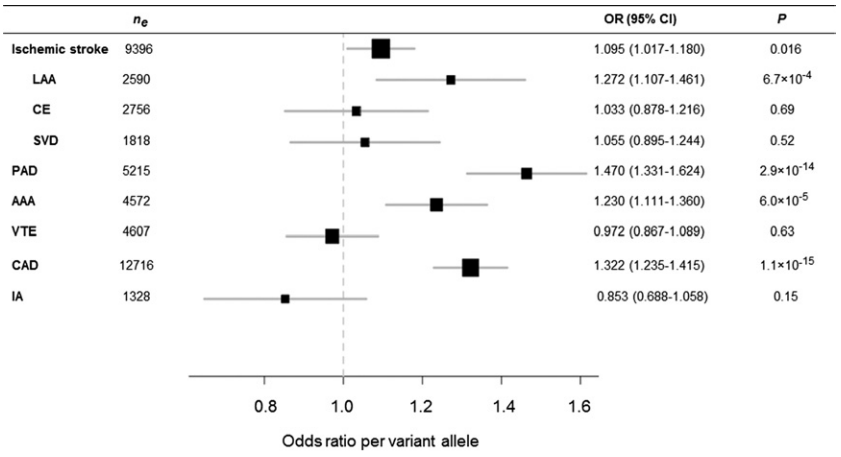

Figure 1 **Association of *LPA* Score With Vascular Diseases**

Forest plot of the associations of apolipoprotein(a) gene (*LPA*) score with ischemic stroke, and TOAST (Trial of Org 10172 in Acute Stroke Treatment) subtypes (large artery atherosclerosis [LAA], cardioembolism [CE], and small vessel disease [SVD]), peripheral arterial disease (PAD), abdominal aortic aneurysm (AAA), venous thromboembolism (VTE), coronary artery disease (CAD), and intracranial aneurysm (IA) in individuals of European origin. Combined odds ratios (ORs) from multiple studies are indicated by squares, with an area in proportion to the sample size, and with horizontal lines representing the 95% confidence intervals (CIs). The plot shows the effective sample sizes (n_e) and p values for the associations.

with both PAD (OR: 1.47; 95% CI: 1.33 to 1.62; $p = 2.9 \times 10^{-14}$) and AAA (OR: 1.23; 95% CI: 1.11 to 1.36; $p = 6.0 \times 10^{-5}$). The previously reported association between CAD and *LPA* score (1) in individuals of European ancestry was confirmed (OR: 1.32; 95% CI: 1.24 to 1.42; $p = 1.1 \times 10^{-15}$). In addition, the *LPA* score was associated with CAD in African Americans (OR: 2.49; 95% CI: 1.08 to 5.72; p = 0.032) (Online Table 4). In contrast, the *LPA* score was not associated with VTE (p = 0.63), IA (p = 0.15) (Fig. 1), or mean common carotid IMT (p = 0.083) (Table 1).

The association of *LPA* score with PAD, LAA, and AAA among patients with and without concomitant CAD. Given the substantial overlap between CAD and the other atherosclerotic diseases, it is conceivable that the associations of the *LPA* variants with LAA stroke, PAD, and AAA are mediated through (or inflated by) the association of these diseases with CAD. Therefore, we reanalyzed the associations between the *LPA* variants and the atherosclerotic phenotypes in cases with and without a history of CAD in studies in which this information was available

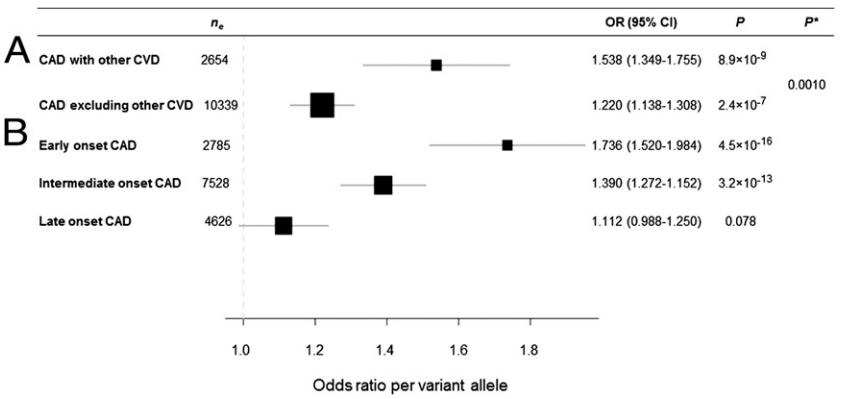

Figure 2 **Association of *LPA* Score With CAD, With and Without Atherosclerosis in Other Vascular Territories, and With Age-Group at Onset of CAD**

(A) Forest plot of the association of *LPA* score with CAD in Iceland, for cases with concomitant cardiovascular disease (CVD; including LAA, PAD, or AAA) and for CAD after excluding cases with known CVD. n_e Values, ORs, 95% CIs and p values are shown. p Values contrasting the 2 groups of CAD cases are shown (*). **(B)** Forest plot of the association of *LPA* score with early onset CAD (diagnosed before the age of 50 years in men and 60 years in women), intermediate onset CAD (diagnosed at the age of 50 to <70 years in men and 60 to <75 years in women), and late onset CAD (diagnosed after age 70 years in men and 75 years in women). Individuals of European origin from Iceland and Atlanta, Georgia, were combined using an inverse variance–weighted meta-analysis approach (fixed-effects model). Abbreviations as in Figure 1.

Table 1 Association of *LPA* Score With Carotid IMT*

Study Group	N	IMT_{log} (mm) β	SE	p Value	p_{het} Value
IMPROVE	2,984	−0.0064	0.0036	0.076	—
Oxfordshire Family Blood Pressure Study	730	−0.0002	0.0224	0.99	—
Combined	3,714	−0.0060	0.0036	0.083	0.70

*Linear regression coefficients (β) with corresponding standard errors (SE) and p values, assuming additive allelic effects. The model included age, sex, body mass index, and physical exercise as covariates. Additional adjustments were made for study site (country) for the IMPROVE study.

IMPROVE = Carotid Intima Media Thickness and IMT-PRogression as Predictors of Vascular Events in a High Risk European Population; IMT = intima-media thickness; *LPA* = apolipoprotein(a) gene; p_{het} = p value for heterogeneity test.

(Online Tables 5A and 5B). After the exclusion of data from patients with CAD, the effect estimates for *LPA* score became lower for PAD (OR: 1.17; p = 0.12) and AAA (OR: 1.11; p = 0.16), suggesting a stronger association of the *LPA* score with atherosclerotic disease manifested in more than 1 vascular bed, although the effect estimate was not lowered for LAA (OR: 1.30; p = 0.013) (Online Table 5A).

Association with CAD, with and without atherosclerosis in other vascular territories. To investigate whether *LPA* score increased the risk for a widespread atherosclerotic disease, we divided the Icelandic CAD cases into those with confirmed atherosclerosis related disease in other vascular beds (PAD, AAA, or LAA), and those without a known history of these diseases. While, for CAD with concomitant PAD, AAA, and/or LAA the OR was 1.54 ($p = 8.9 \times 10^{-9}$), the OR for CAD without known PAD, AAA, and/or LAA was 1.22 ($p = 2.4 \times 10^{-7}$). On direct comparison of the 2 groups of CAD cases, each *LPA* risk allele increased the risk for atherosclerosis in other vascular beds by 26% (p = 0.0010) (Fig. 2).

Association with angiographic extent of CAD and with MI among patients with angiographic CAD. Online Table 6 shows the distribution of the number of affected (>50% stenosis) coronary index vessels, and the proportions with MI among 5,588 subjects who had undergone coronary angiography. After adjusting for sex, age at angiography, study site, and ethnicity, in a linear regression model, each *LPA* risk allele increased the number of diseased vessels by a mean of 0.267 ($p = 4.8 \times 10^{-12}$). Effect estimates for African Americans were similar to those of patients of European origin (Table 2). In a logistic regression model comparing patients with angiographic CAD with MI (n = 1,817) or without MI (n = 1,908), the *LPA* score was not associated with MI after adjusting for the same variables and the age at first CAD diagnosis (OR: 0.99; p = 0.90).

Table 2 Association of *LPA* Score With Angiographic CAD Severity*

Study Group	N	No. of Affected Coronary Index Vessels β	SE	p Value
Iceland	2,330	0.245	0.061	5.8×10^{-5}
Atlanta-European American	2,718	0.281	0.052	6.3×10^{-8}
Atlanta-African American	540	0.396	0.219	0.071
Combined	5,588	0.267	0.038	4.8×10^{-12}

*Linear regression coefficients (β) with corresponding standard errors (SE) and p values, assuming additive allelic effects. Age at angiography and sex were included as covariates in the model for each study group. In the combined analysis additional adjustments were made for study group (site/ethnicity).

CAD = coronary artery disease; *LPA* = apolipoprotein(a) gene.

Table 3 Association Between *LPA* Score and the Age at Diagnosis of CAD*

Parameter	n	Age at Diagnosis β	SE	p Value
European ancestry				
Age at first CAD diagnosis	9,275	−1.581	0.295	8.2×10^{-8}
Age at first MI	5,148	−0.918	0.416	0.028
Age at first CAD diagnosis for MI cases	5,148	−1.334	0.400	0.00085
African Americans				
Age at first CAD diagnosis	333	−3.541	2.521	0.16
Age at first MI	185	−2.221	3.249	0.50
Age at first CAD diagnosis for MI cases	185	−6.009	3.159	0.059

*Linear regression coefficients (β) with corresponding standard errors (SE) and p values, assuming additive allelic effects. The model included sex as a covariate. In addition, study site (Iceland/Atlanta) was a covariate in the model when analyzing those of European ancestry.

MI = myocardial infarction. Other abbreviations as in Table 2.

Association with age of onset of CAD and MI. The correlation between *LPA* score and the age at diagnosis of CAD was tested, with adjustments for sex and study site (Table 3, Fig. 2). Each *LPA* risk allele was associated with a mean of 1.58 years' earlier diagnosis of CAD ($p = 8.2 \times 10^{-8}$) among 9,276 cases of European origin from Iceland and Atlanta, Georgia (Online Table 4). Restricting data to those from cases with MI diagnoses, the corresponding reduction in age at diagnosis of first MI was 0.92 years (p = 0.028). Effect estimates for age at first CAD diagnosis among 333 African Americans were consistent with those observed for whites, but did not reach nominal significance levels.

Discussion

We report an association between 2 Lp(a)-related SNPs (combined and measured as *LPA* score), and the PAD, AAA, and LAA subtype of ischemic stroke, with each variant allele increasing the risk by 47%, 23%, and 27% for the 3 diseases, respectively. In contrast, our analyses show no association between the *LPA* variants and VTE or CE and SVD stroke subtypes. We show that patients with CAD carrying *LPA* risk alleles have increased susceptibility to atherosclerotic manifestations outside of the coronary tree and are more likely to be diagnosed earlier with CAD than are CAD cases not carrying these variants. Further, our study provides evidence for an association between the *LPA* variants and the number of coronary arteries (out of 4 index

vessels) with >50% stenosis. Thus the *LPA* variants, and by implication, Lp(a) levels, have an effect on the atherosclerotic burden of large vessels throughout the arterial tree. To the best of our knowledge, relationships between sequence variants affecting Lp(a) levels and either systemic distribution of atherosclerosis or angiographic CAD severity, have not been reported before. However, several studies, that in general have been limited by small sample sizes, have assessed the association between Lp(a) levels and severity of CAD and provided conflicting results (26–30).

In apparent contrast to the correlation between *LPA* score and earlier age at onset of CAD, *LPA* score was not associated with IMT, a marker for subclinical atherosclerosis (31,32). This is consistent with results from a recent population-based cohort study showing that Lp(a) levels (and SNPs correlated with Lp[a]), were not associated with IMT (33), and in line with a recent meta-analysis of genome-wide association studies from the CHARGE (Cohorts for Heart and Aging Research in Genomic Epidemiology) consortium (34) that found little evidence for genetic overlap across carotid IMT and CAD. We suggest that the effects of the *LPA* variants, and thus Lp(a) levels, on the risk for atherosclerotic manifestations are mediated at later stages in the pathogenic pathway and are thus not reflected by variation in carotid IMT.

Our study was limited by the fact that measurements of Lp(a) levels were not available, rendering it impossible to show directly that the association of the *LPA* risk variants with atherosclerotic phenotypes is mediated through Lp(a) levels. Therefore, the possibility that the risk conferred by the *LPA* score is mediated through mechanisms other than increased Lp(a) levels, cannot be disregarded. However, previous studies have shown a strong and consistent association between the 2 *LPA* variants and Lp(a) levels (1,35), and the association between the *LPA* risk variants and CAD has been shown to disappear with Lp(a) level adjustments (1), strongly supporting the view that the effect on atherosclerotic phenotypes is mediated through Lp(a) levels.

Lp(a) has been implicated in both atherogenesis and thrombosis. We show that *LPA* risk alleles that correlate with high plasma Lp(a) levels were associated with vascular diseases with a strong atherosclerotic component, such as CAD, PAD and LAA, and AAA, but we failed to demonstrate an association between *LPA* alleles and diseases without an atherosclerotic etiology, such as VTE and IA, or diseases less related to atherosclerosis, such as the CE and SVD stroke subtypes. Furthermore, among patients with angiographic CAD, after adjustments for age at onset of CAD, the *LPA* variants were not associated with MI, indicating that the risk is mediated through atherosclerotic plaque deposition, rather than plaque rupture, or subsequent thrombosis. Given the assumption that the risk for *LPA* variants on vascular diseases is mediated through Lp(a) levels, our results suggest that the implicit thrombogenic properties of Lp(a) (4,5) are insufficient to increase the risks for the thrombotic stroke subtypes CE and SVD, or to promote VTE.

Conclusions

We report an association between 2 variants in the *LPA* gene and 3 atherosclerosis-related diseases (i.e., the LAA subtype of ischemic stroke, PAD, and AAA). In addition to replicating the previously reported association between the *LPA* variants and CAD, we report a correlation between the *LPA* variants and the number of obstructed coronary arteries. Furthermore, the risk variants were associated with an earlier diagnosis of CAD. Evaluating *LPA* risk alleles may therefore contribute to more effective primary and secondary prevention of atherosclerotic disease. In contrast, we found no association with the CE and SVD ischemic stroke subtypes, which have less obvious atherosclerotic components, or VTE, a primarily thrombotic disease. These findings imply that the risk conferred by high Lp(a) levels is mediated through the atherosclerotic, rather than the thrombotic, aspects of vascular disease.

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Key Words: association ■ atherosclerosis ■ genetic ■ lipoprotein(a) ■ thrombosis.

APPENDIX:

For supplementary tables and text, please see the online version of this article.