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Conventional and Mendelian randomization analyses suggest no association between lipoprotein(a) and early atherosclerosis: the Young Finns Study

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Background Lipoprotein(a) [Lp(a)] is an established risk factor for coronary disease and stroke, but mechanisms underlying this association are unknown. We examined the association of Lp(a) with early atherosclerosis by using conventional epidemiologic analysis and a Mendelian randomization analysis. The latter utilized genetic variants that are associated with Lp(a) to estimate causal effect.

Methods A prospective population-based cohort study of 939 men and 1141 women was conducted. Lp(a) was measured repeatedly at mean ages 17 and 38 years. Measurements of carotid intima-media thickness (IMT) and brachial flow-mediated dilation (FMD) at mean ages 32 and 38 years were used to determine the level and 6-year progression of subclinical atherosclerosis. Lp(a)-related genetic variant, rs783147, was identified by a genome wide association analysis ($P = 3.1 \times 10^{-58}$), and a genetic score was constructed based on 10 Lp(a)-related variants. Mendelian randomization test was performed using a two-stage instrumental variables analysis.

Results rs783147 and the genetic score were strong instruments for nonconfounded Lp(a) levels (F-statistics 269.6 and 446.0 in the first-stage instrumental variable analysis). However, Lp(a) levels were not associated with the levels of or change in IMT or FMD in any of the conventional and instrumental variables tests. The null finding was observed both with rs783147 and the genetic score as instruments and remained unchanged after adjustment for clinical characteristics, such as age, sex, HDL and LDL cholesterol, ApoB, systolic and diastolic blood pressure, diabetes and smoking.

Conclusions Data from conventional and Mendelian randomization analyses provide no support for early atherogenic effects of increased Lp(a) levels.

Keywords Lipoprotein(a), atherosclerosis, risk factors, single-nucleotide polymorphism, Mendelian randomization

Introduction

Lipoprotein(a), or Lp(a), is composed of a low-density lipoprotein (LDL)-like particle covalently bound to a glycoprotein, apolipoprotein(a).[1] Lp(a) contains cholesterol, cholesteroesters, phospholipids, triglycerides and apolipoprotein B (ApoB).[1] The physiological function and cardiovascular effects of Lp(a) are poorly understood, but several epidemiological studies have shown high Lp(a) concentration to be associated with an increased risk of coronary disease and stroke.[2,3] Furthermore, recent evidence from single-nucleotide polymorphisms (SNPs) affecting Lp(a) levels suggests that the association with coronary disease may be causal.[4–8]

Atherosclerosis is a major component of coronary disease and stroke aetiology, but whether the effect of Lp(a) on these vascular endpoints is mediated through accelerated atherosclerosis remains unclear. The few available studies published on this issue have failed to demonstrate a robust link between Lp(a) and indicators of atherosclerosis, such as intima-media thickness (IMT) and brachial flow-mediated arterial dilation (FMD).[9–15] Confounding may have contributed to inconsistencies in epidemiologic evidence. A Mendelian randomization approach exploits genetic variants related to hypothesized risk factors to improve causal inference when using observational data.[16] However, to our knowledge, no Mendelian randomization analyses using *LPA* SNPs as instruments for non-confounded Lp(a) levels are available for these atherosclerosis outcomes.

In this study, we therefore use both conventional and Mendelian randomization analyses to examine whether Lp(a) concentrations and Lp(a)-related genetic variants are associated with levels and progression of early atherosclerosis in a large population-based cohort.[17]

Methods

Study population and genotyping

The study population and methods have been described previously.[18,19] Briefly, the subjects were 939 white men and 1141 white women with data on *LPA*-genotype and atherosclerosis from the population-based Young Finns cohort study. The subjects were genotyped with custom-made Illumina 670K chip according to manufacturer's protocols. Genotypes were culled from intensity data using Illuminus software.[20] In genome wide analysis, we identified several variants near the known *LPA* locus at chromosome 6q26–27 that were strongly associated with Lp(a) levels. We chose rs783147 (chromosome 6: 161 057 980) in the *LPA* gene as the relevant genetic marker for this study because it had the strongest association with Lp(a) levels ($P = 3.1 \times 10^{-58}$). For a subsidiary analysis, we selected 20 SNPs with a strong association with Lp(a) levels (the 20 lead SNPs in the genome wide analysis; see Table 1 in supplementary data available at *IJE* online).

Assessment of Lp(a)

Lp(a) was measured in serum stored at −70°C. In 2007, we used immunoturbidimetric method after <6 months' storage (Lp(a)-HA reagent, Wako Chemicals GmbH, Germany). The detection limit for this method is 1.0 mg/dl and the antibody used in the reagent is reactive to the various apolipoprotein(a) isoforms.[21] In 1986, Lp(a) was assessed by radioimmunoassay (detection limit 3.0 mg/dl), according to the same principles as described for an immunoenzymatic assay,[22] using research kits from Pharmacia Diagnostics, Uppsala, Sweden.

Indicators of atherosclerosis

We performed ultrasound studies in 2001 and 2007 using Sequoia 512 ultrasound mainframes (Acuson, CA, USA) with 13.0-MHz linear array transducers to assess carotid IMT and brachial FMD.[18,19] Carotid IMT, used as a surrogate marker for atherosclerosis, correlates with cardiovascular risk factors[19] and the severity of coronary atherosclerosis[23] and is a predictor of future vascular events, such as stroke and myocardial infarction.[24] We measured carotid IMT on the posterior (far) wall of the left carotid artery. At least four measurements were taken ~10 mm proximal to the bifurcation to derive mean carotid IMT. At this region of the common carotid artery, none of the subjects had plaques. The digitally stored scans were manually analysed by the same reader in 2001 and 2007 blinded to subjects' details. The between-visit coefficient of variation (CV) of IMT measurements was 6.4%, and the intra-observer CV was 3.4%.

Brachial FMD is a correlate of coronary endothelial function[25] and there is some evidence to suggest an association between FMD and subsequent progression of carotid IMT,[26] consistent with the model that

Table 1 Characteristics of study participants

Characteristic[a]	*N*	Statistic[b]
Age, years	2080	37.7 (5.0)
Sex, %		
Male	939	45.1
Female	1141	54.9
LPA SNP (rs783147)		
AA (CC)	595	28.6
AB (CT)	1044	50.2
BB (TT)	441	21.2
Lp(a), mg/dl[c]	1670	7.5 (3.3–18.5)
Lp(a) in 1986, mg/dl[c]	1593	4.5 (1.5–14.3)
Carotid IMT, mm	2014	0.626 (0.096)
Carotid IMT in 2001, mm	2080	0.581 (0.092)
ΔIMT (2001 to 2007), mm	1664	0.046 (0.084)
FMD, %	1934	7.98 (4.38)
FMD in 2001, %	1654	8.77 (4.57)
ΔFMD (2001 to 2007), %	1534	0.86 (5.40)
HDL-C, mmol/l	2001	1.34 (0.33)
LDL-C, mmol/l	1973	3.07 (0.78)
ApoA1, g/l	2010	1.60 (0.26)
ApoB, g/l	2010	1.02 (0.26)
Systolic blood pressure, mmHg	2009	121 (14)
Diastolic blood pressure, mmHg	2008	76 (11)
BMI, kg/m^2	1982	26.0 (4.7)
Diabetes mellitus, %		
No	1965	98.5
Yes	31	1.5
Current smoking		
No	1630	81.6
Yes	368	18.4

[a]Measured in 2007 unless otherwise stated.
[b]Statistics are mean (SD) for continuous variables or percentage for categorical variables unless otherwise stated.
[c]Median (inter-quartile range).

endothelial function becomes abnormal before the structural changes in intima-media occur. We measured the left brachial artery diameter at rest and during reactive hyperaemia. Increased flow was induced by inflation of a pneumatic tourniquet placed around the forearm to a pressure of 250 mmHg for 4.5 min, followed by a release. Three measurements of arterial diameter were performed at end-diastole at a fixed distance from an anatomic marker at rest and 40, 60 and 80 s after cuff release. The vessel diameter after reactive hyperaemia was expressed as the percentage relative to resting diameter. The greatest value between 40 and 80 s was used to derive FMD. The 3-month between-visit CV was 3.2% for diameter measurements and 26.0% for FMD. All FMD measurements were performed by one experienced reader.

Assessment of risk factors

Other clinical characteristics were assessed in 2007 using standard procedures: HDL and LDL cholesterol (from Friedewald formula), apolipoproteins A1 (ApoA1) and ApoB, systolic and diastolic blood pressure, body mass index (BMI), smoking status and prevalent diabetes mellitus.

Statistical analysis

We used the PLINK toolset (http://pngu.mgh.harvard.edu/purcell/plink/)[27] to identify SNPs associated with Lp(a) levels and performed all other statistical analyses with Stata, version 10 (Stata Institute, Texas, USA). In order to maximize statistical power, each analysis was based on the maximum number of subjects. Lp(a) values below the detection limit were assigned a value of 0.5 mg/dl in 2007 (4.6%) and 1.5 mg/dl in 1986 (36.0%). Lp(a) levels were $\log_e$-transformed before the analysis to reduce skewness. Age- and sex-adjusted associations of rs783147 and Lp(a), as exposures, with levels of and change in IMT and FMD, as outcome measures, were studied using linear regression analysis. Multivariable models of the association between Lp(a) and these outcomes were additionally adjusted for HDL and LDL cholesterol, ApoB, systolic and diastolic blood pressure, diabetes and smoking. We corrected LDL cholesterol and ApoB levels for the Lp(a) contribution prior to their inclusion in the analysis.[28] Thus, total lipoprotein(a) mass was multiplied by 0.3, and this value was subtracted from LDL cholesterol values. Similarly, total lipoprotein(a) mass multiplied by 0.16 was subtracted from ApoB values.

In addition to conventional linear regression analysis, we performed an instrumental variables regression analysis to examine the association of Lp(a) with IMT and FMD in a Mendelian randomization study.[16,29] Mendelian randomization is used to estimate the magnitude of a causal effect of a risk factor on an outcome by using genetic variants as instrumental variables. Because the genotype, being determined at conception, necessarily precedes the onset of atherosclerosis, any association of the genetic variant with IMT and FMD should be unaffected by reverse causation bias arising from the potential effects of atherosclerosis on Lp(a) concentration. Furthermore, the random assortment of alleles at the time of gamete formation leads to population distributions of genetic variants that are generally independent of the environmental exposures that commonly confound epidemiological risk factor–disease associations.

The first stage of the instrumental variable analysis tested the association between the genetic variant

Table 2 Age- and sex-adjusted association of clinical characteristics and *LPA* polymorphism rs783147 with carotid IMT and brachial flow-mediated dilation

	IMT, mm			FMD, %		
Characteristic[a]	*N*	Regression coefficient (SE)	*P*	*N*	Regression coefficient (SE)	*P*
HDL-C, mmol/l	2001	−0.038 (0.006)	<0.0001	1986	−0.125 (0.320)	0.70
LDL-C, mmol/l	1973	0.009 (0.003)	0.001	1958	0.207 (0.134)	0.12
ApoA1, g/l	2010	−0.033 (0.008)	<0.0001	1995	0.236 (0.406)	0.56
ApoB, g/l	2010	0.053 (0.008)	<0.0001	1995	1.129 (0.402)	0.005
Systolic blood pressure, mmHg	2009	0.001 (0.0001)	<0.0001	1994	−0.000 (0.007)	0.95
Diastolic blood pressure, mmHg	2008	0.001 (0.0002)	<0.0001	1993	0.007 (0.009)	0.44
BMI, kg/m^2	1982	0.005 (0.0004)	<0.0001	1967	0.098 (0.021)	<0.0001
Diabetes mellitus, 0 = no, 1 = yes	1996	0.038 (0.016)	0.02	1981	0.072 (0.802)	0.93
Current smoking, 0 = no, 1 = yes	1998	0.006 (0.005)	0.25	1983	−0.238 (0.259)	0.34
LPA SNP, per allele	2014	−0.000 (0.003)	0.93	1999	0.115 (0.141)	0.42

[a]All variables were measured in 2007.

rs783147 (the instrument) and Lp(a). *F* statistics were used to evaluate the strength of the instrument, with values greater than 10 taken as evidence against weak instruments.[30,31]

The second stage tested the association between Lp(a) (as instrumented by rs783147) with IMT and FMD. We compared results from the instrumental variable estimates of this association to those from standard linear regression using the Durbin form of the Durbin-Wu-Hausman statistic.[32] To test the robustness of the instrumental variable estimates, we repeated the analysis by using a simple additive genetic risk score based on 10 and 20 SNPs as instruments for Lp(a) levels.

Results

Clinical characteristics of the study population are presented in Table 1. The genotype frequencies in rs783147 were in Hardy–Weinberg equilibrium ($P > 0.6$), thus supporting the integrity of our genetic data. Minor allele frequency (MAF) was 0.47. After adjustment for age and sex, rs783147 was modestly associated with ApoB (regression coefficient B per A allele = 0.017, $P = 0.04$) and HDL cholesterol ($B = -0.019$, $P = 0.05$) but unrelated to LDL cholesterol ($B = 0.036$, $P = 0.14$), ApoA1 ($B = -0.007$, $P = 0.38$), systolic blood pressure ($B = -0.675$, $P = 0.11$), diastolic blood pressure ($B = -0.405$, $P = 0.24$), BMI ($B = 0.064$, $P = 0.67$), diabetes ($B = 0.002$, $P = 0.67$) and current smoking ($B = -0.007$, $P = 0.58$).

Mean carotid IMT was 0.64 [standard deviation (SD) 0.11] mm in men and 0.61 (SD 0.08) mm in women. In an age- and sex-adjusted analysis, HDL and LDL cholesterol, ApoA1 and ApoB, systolic and diastolic blood pressure, BMI and diabetes were all associated with IMT. Mean FMD was 7.6% (SD 3.8) in men and 9.9% (SD 4.9) in women. Only ApoB and BMI were associated with FMD. SNP rs783147 was not associated with IMT or FMD (Table 2).

Conventional regression analysis

As expected, logLp(a) was associated with LDL cholesterol [$\beta = 0.095$, standard error (SE) = 0.016, $P < 0.0001$] and ApoB ($\beta = 0.014$, SE = 0.005, $P = 0.009$). However, both age- and sex-adjusted and multivariable adjusted conventional regression analyses provided null findings for all associations of logLp(a) with IMT (Table 3) and FMD (Table 4). As residual confounding in conventional regression analyses can lead to an underestimation of true associations, we continued to examine the association between Lp(a) and indicators of atherosclerosis by using Mendelian randomization.

Instrumental variables analysis using rs783147 as an instrument

There was a strong association between rs783147 and Lp(a) levels, with median (IQR) values being 3.3 (1.5–5.3), 7.3 (3.9–16.7) and 13.1 (5.8–28.1) mg/dl for those with 0, 1 and 2 effect alleles respectively. SNP rs783147 explained 11.7% of the variance in logLp(a). *F*-values of 269.6 and 37.59 in the first-stage regression analysis suggest that rs783147 is a strong instrument for Lp(a) levels in 2007 and in 1986.

Table 3 Association of Lp(a) with carotid IMT according to standard regression analysis and *LPA* polymorphism rs783147 instrumented regression analysis

Exposure and analytic approach	Outcome	*N*	Regression coefficient (SE)	*P*
LogLp(a) lipoprotein in 1986, mg/dl	IMT in 2001, mm			
Standard regression, age and sex adjusted		1593	0.003 (0.002)	0.09
Instrumental variables regression, age and sex adjusted		1593	−0.000 (0.006)	0.96
LogLp(a) lipoprotein in 1986, mg/dl	IMT in 2007, mm			
Standard regression, age and sex adjusted		1540	0.001 (0.002)	0.46
Instrumental variables regression, age and sex adjusted		1540	−0.000 (0.007)	0.95
LogLp(a) lipoprotein in 1986, mg/dl	ΔIMT (2001–07), mm			
Standard regression, age and sex adjusted		1308	−0.000 (0.002)	0.99
Instrumental variables regression, age and sex adjusted		1308	−0.008 (0.007)	0.26
LogLp(a) lipoprotein in 2007, mg/dl	IMT in 2007, mm			
Standard regression, age and sex adjusted		2010	0.001 (0.002)	0.68
Standard regression, multivariable adjusted[a]		1918	0.000 (0.002)	0.82
Instrumental variables regression, age and sex adjusted		2010	−0.000 (0.002)	0.91
Instrumental variables regression, multivariable adjusted[a]		1918	−0.000 (0.005)	0.95

[a]Adjusted for age, sex, HDL and LDL cholesterol, ApoB, systolic and diastolic blood pressure, diabetes and smoking. Correction for the cholesterol content of Lp(a) particles was made by subtracting estimated Lp(a) cholesterol values from the levels of LDL cholesterol and ApoB values prior to inclusion in the analysis.

Table 4 Association of Lp(a) with brachial FMD according to standard regression analysis and *LPA* polymorphism rs783147 instrumented regression analysis

Exposure and analytic approach	Outcome	*N*	Regression coefficient (SE)	*P*
LogLp(a) lipoprotein in 1986, mg/dl	FMD in 2001, %			
Standard regression, age and sex adjusted		1492	0.086 (0.092)	0.35
Instrumental variables regression, age and sex adjusted		1492	0.229 (0.329)	0.49
LogLp(a) lipoprotein in 1986, mg/dl	FMD in 2007, %			
Standard regression, age and sex adjusted		1530	−0.148 (0.097)	0.13
Instrumental variables regression, age and sex adjusted		1530	0.115 (0.335)	0.73
LogLp(a) lipoprotein in 1986, mg/dl	ΔFMD (2001–07), % units			
Standard regression, age and sex adjusted		1215	−0.227 (0.131)	0.08
Instrumental variables regression, age and sex adjusted		1215	−0.077 (0.466)	0.87
LogLp(a) lipoprotein in 2007, mg/dl	FMD in 2007, %			
Standard regression, age and sex adjusted		1995	−0.051 (0.082)	0.53
Standard regression, multivariable adjusted[a]		1903	−0.082 (0.082)	0.33
Instrumental variables regression, age and sex adjusted		1995	0.178 (0.237)	0.45
Instrumental variables regression, multivariable adjusted[a]		1903	0.224 (0.241)	0.35

[a]Adjusted for age, sex, HDL and LDL cholesterol, ApoB, systolic and diastolic blood pressure, diabetes and smoking. Correction for the cholesterol content of Lp(a) particles was made by subtracting estimated Lp(a) cholesterol values from the levels of LDL cholesterol and ApoB values prior to inclusion in the analysis.

The second-stage instrumental variables analysis, using rs783147 as an instrument for deconfounded Lp(a) levels, found no evidence of an association of Lp(a) with IMT or FMD (Tables 3 and 4). Instrumental-variable estimates of the association of Lp(a) with IMT and FMD did not differ from those obtained from conventional regression analyses ($P > 0.31$ in age- and sex-adjusted models)

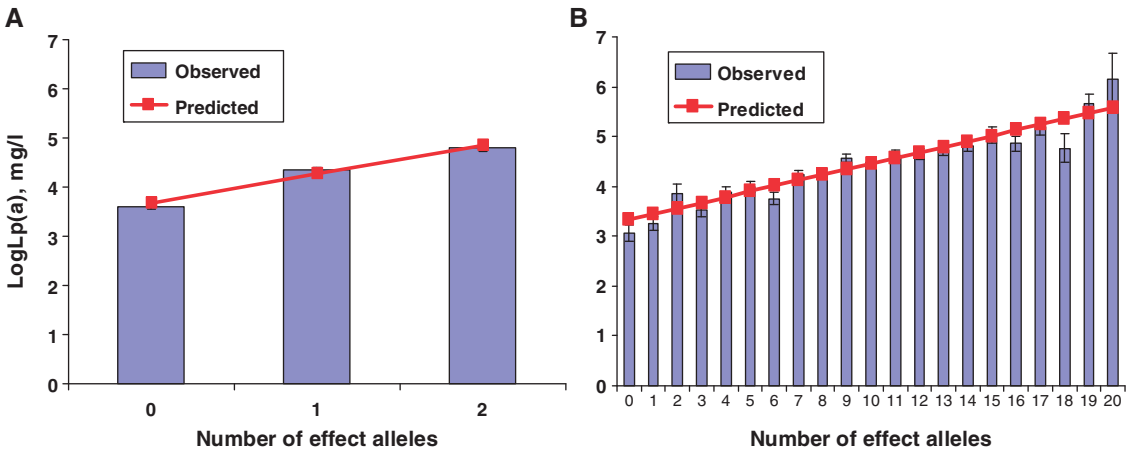

Figure 1 Associations of rs783147 (A) and genetic score from 10 SNPs (B) with logarithmically transformed lipoprotein(a) levels

Instrumental variables analysis using genetic score as an instrument

A genetic score based on 10 SNPs (an additive model) explained 17.5% of the variance in logLp(a). *F*-values of 446.0 and 72.4 from the first-stage regression suggest that the genetic score was a stronger instrument for Lp(a) in 2007 and 1986 than rs783147 alone. Figure 1 illustrates this with predicted means (linear regression) and observed (genotype specific) means of logLp(a) in 2007 by number of effect alleles in rs783147 (Figure 1A) and the number of effect alleles in the genetic score (Figure 1B).

In agreement with the rs783147-based analyses, instrumental variables regression with the genetic score provided no support for an association of Lp(a) with IMT or FMD (Table 2 in supplementary data available at *IJE* online). No further analyses were performed using the first 20 SNPs as they did not provide a stronger genetic instrument [$F = 353.98$ and 57.10 for Lp(a) in 2007 and 1986, respectively] than the 10-SNP score.

Discussion

This population-based cohort study exploiting both phenotypical and genotype data found no association between Lp(a) and early atherosclerosis, as indicated by carotid IMT and brachial flow-mediated dilation. We are not aware of previous Mendelian randomization data on this issue, but our conventional analyses are in line with prior small-scale studies employing only a single measurement of Lp(a) and atherosclerosis.[9–15] In the Atherosclerosis Risk in Communities (ARIC) study, Lp(a) was associated with IMT, but only in subgroups, such as white men and diabetic black women.[15]

Some limitations to this study and the Mendelian randomization method may contribute to false null findings. First, measurement error reduces opportunities to detect an association and is considered to be greater for Lp(a) levels determined based on frozen rather than fresh samples[33] and assays sensitive to apolipoprotein(a) isoforms.[34] In this study, Lp(a) was measured in 2007 using apolipoprotein(a) isoform independent assays and we found a strong association between *LPA* genetic variants (which are not affected by this source of error) and Lp(a), supporting the accuracy of our measurement.

Second, population stratification, pleiotropic effects of the genetic variant, and an association of the genetic instrument with other genetic variants related to the outcome can potentially bias Mendelian randomization analysis.[16,35] These are not likely explanations for our findings given that our cohort included only White Europeans and there was no strong evidence of associations between the *LPA* SNPs and potential confounding factors. Developmental canalization (i.e. genetic variants inducing compensatory phenomena that mask the effect on the outcome) is a source of bias in Mendelian randomization, but seems an implausible explanation for our findings, because it would mean that the canalization masks the effect on atherosclerosis but not on vascular disease endpoints such as myocardial infarction and stroke.[4–8]

Third, as influences of genetic variants on risk factors are typically small, Mendelian randomization estimates of associations tend to be less precise (although they are also assumed to be less biased) than estimates from conventional regression analysis. In this study, a genome wide association analysis allowed identification of strong instruments for modelling Lp(a) levels. Indeed, *F*-values of >400 in the first-stage regression are exceptionally high, being 5–40 times higher than those reported in several previous Mendelian randomization studies with positive findings for other risk factors.[36–38] Nevertheless, as indicated by large standard errors of the estimates, the present study is not well

powered to detect small effects of Lp(a) on atherosclerosis; meta-analyses that pool our data with findings from future Mendelian randomization studies are needed to detect such effects.

The validity of the present data is supported by the convergent results across different analyses, repeated measurements of Lp(a) (with two different methods), and more than one validated indicator for atherosclerosis. A recent meta-analysis of 36 prospective studies found Lp(a) to be an independent, albeit modest, predictor of coronary disease and stroke mortality.[2] Large-scale studies have also confirmed an association of genetic variants influencing levels of Lpa(a) with these diseases.[4–8] However, subclinical atherosclerosis does not equate future cardiovascular event risk,[39] and *in vitro* and animal studies have reported that Lp(a) may in particular promote thrombosis and inflammation,[40,41] i.e. factors that are associated with coronary events and stroke but do not necessarily causally contribute to subclinical atherosclerosis in early adulthood.

In conclusion, all the tests performed in this study suggest that higher Lp(a) concentration does not promote early atherosclerotic changes in a clinically meaningful way among young adults. These findings need to be replicated in other independent cohorts. Our evidence suggests that future research should also be directed to other potential pathological mechanisms, such as prothrombotic and proinflammatory effects and progression of atherosclerosis in later life.

Supplementary data

Supplementary data are available at *IJE* online.

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Conflict of interest: None declared.

KEY MESSAGES

- High lipoprotein(a) [Lp(a)] concentration is associated with an increased risk of coronary disease and stroke. Whether these effects are mediated through accelerated atherosclerosis remains unclear.
- In a prospective study of 2080 Finns, Lp(a) concentration was not associated with the levels of, or change in, carotid intima-media thickness (IMT) or brachial flow-mediated dilation (FMD) in adulthood.
- Single-nucleotide polymorphism rs783147 provided a strong instrument for Lp(a) levels. In spite of this, Mendelian randomization analyses provided null findings for all associations of Lp(a) with carotid IMT or FMD.
- These data suggest that Lp(a) concentration does not contribute to early atherosclerotic changes in a clinically meaningful way.

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Commentary: Lipoprotein(a) and atherosclerosis

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Recently, there has been a resurgence of interest in lipoprotein(a) [Lp(a)] as a risk factor for atherosclerotic disease. Lp(a) was first discovered by Kaare Berg in 1962 on the basis of antigenic differences in low-density lipoprotein (LDL) cholesterol detected after injecting rabbits with human LDL particles.[1] The resulting antibodies identified an antigen in some, but not in other individuals. In the early 1970s, Berg reported that Lp(a) was a plasma lipoprotein consisting of an LDL particle linked with an apolipoprotein(a) particle. The size of the LDL particle was fixed, but the apolipoprotein(a) component varied between individuals. The inheritance of Lp(a) was clarified in 1987 by Gerd Utermann, who identified multiple size variants that were strongly associated with plasma levels of Lp(a).[2] But the causal relevance of Lp(a) for coronary disease and for other vascular diseases remained uncertain for almost 50 years after the initial discovery.

In 2009, three large studies reported that genetic variants encoding plasma levels of Lp(a) had higher risks of coronary heart disease.[3–5] These Mendelian randomization studies examined the associations of genetic variants encoding plasma levels of Lp(a), plasma levels of Lp(a) and coronary disease. In the PROCARDIS study, we examined 27 single nucleotide polymorphism (SNPs) at the *LPA* locus at 6q26-27, and found two SNPs (rs10455872 and rs3798220) that were strongly and independently related to coronary disease.[5] We replicated these associations in a meta-analysis involving a total of 8000 coronary cases. Both *LPA* SNPs explained about half the genetic variation in plasma levels of Lp(a) and their effect on risk of coronary disease was completely attenuated after adjustment for plasma levels of Lp(a), providing strong support for causality.[5]

However, the mechanism by which elevated plasma levels of Lp(a) cause vascular disease remains uncertain. In a Mendelian randomization study reported in this issue, Kivimaki and colleagues examined the associations of *LPA* variants; and Lp(a) levels with carotid intima–media thickness and brachial artery flow-mediated dilatation.[6] In a population-based study of almost 2000 healthy Finnish individuals, Kivimaki demonstrated no association of a variant at the *LPA* locus (rs783147), that was strongly associated with Lp(a) levels, with either of these markers of early atherosclerosis. The results of this study require corroboration by larger studies (and meta-analyses of such studies), and with a genotype score involving SNPs associated with greater differences in Lp(a) levels, but the available evidence does not suggest any role for Lp(a) in early atherosclerosis.

Whereas the mechanism by which elevated levels of Lp(a) cause coronary disease remains elusive, the Mendelian randomization studies of *LPA* variants and coronary disease have prompted a paradigm shift in the importance of Lp(a) as a risk factor for coronary disease. Further large-scale studies are required to assess the relevance of *LPA* variants for stroke and other vascular diseases. The results of