Clinical and Population Studies

Genetic Evidence That Lipoprotein(a) Associates With Atherosclerotic Stenosis Rather Than Venous Thrombosis

Pia R. Kamstrup, Anne Tybjærg-Hansen, Børge G. Nordestgaard

Objective—The aim of the present study was to determine whether lipoprotein(a) [Lp(a)], considered a causal risk factor for cardiovascular disease, primarily promotes thrombosis or atherosclerosis.

Methods and Results—Using a Mendelian randomization study design, we measured plasma Lp(a) and genetically elevated Lp(a) levels through the LPA kringle IV type 2 repeat genotype in 41,231 individuals. We included 2 general population studies of both venous thrombosis and combined thrombosis and atherosclerosis in coronary arteries (myocardial infarction), and 3 case–control studies of atherosclerotic stenosis. Neither Lp(a) tertiles nor LPA kringle IV type 2 tertiles associated with the risk of venous thrombosis in general population studies (trend: P=0.12–0.76), but did each associate with risk of coronary, carotid, and femoral atherosclerotic stenosis in case–control studies (trend: P<0.001 to 0.04). Lp(a) and LPA kringle IV type 2 tertiles also associated with the risk of myocardial infarction in general population studies (trend: P<0.001 to 0.003). For doubling of Lp(a) levels, instrumental variable estimates of hazard/odds ratios were 1.02 (95% CI 0.90–1.15) and 1.04 (0.93–1.16) for venous thrombosis in the 2 general population studies, 1.12 (1.01–1.25), 1.17 (1.05–1.32), and 1.16 (1.01–1.35), respectively, for coronary, carotid, and femoral atherosclerotic stenosis in case–control studies, and 1.21 (1.10–1.33) and 1.17 (1.05–1.29) for myocardial infarction in general population studies.

Conclusion—This supports that Lp(a) primarily promotes atherosclerotic stenosis rather than venous thrombosis. (Arterioscler Thromb Vasc Biol 2012;32:1732–1741.)

Key Words: cardiovascular disease ■ epidemiology ■ genetics ■ lipoproteins ■ mechanism

Prospective epidemiological studies demonstrate a robust and specific association between elevated lipoprotein(a) [Lp(a)] levels and increased risk of cardiovascular disease.1–5 This, together with the results from recent genetic studies, including our own study demonstrating an association of LPA genotypes with risk of myocardial infarction, strongly supports Lp(a) as a direct cause of cardiovascular disease.6–7 Despite ample data from conventional mechanistic studies demonstrating both prothrombotic and proatherosclerotic effects of Lp(a), it is, however, still unclear whether Lp(a) promotes cardiovascular disease primarily via increased thrombosis or atherosclerosis. This remains an important question to solve to better target preventive treatment for high Lp(a) levels. We here present results of a genetic epidemiological approach to help resolve the question.

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Lp(a) consists of a cholesterol-laden low-density lipoprotein (LDL)-particle bound to a glycoprotein, apolipoprotein(a) [apo(a)], that structurally resembles plasminogen.8 Apo(a) is composed of a large and variable number of plasminogen-like kringle IV (KIV) structures, 1 plasminogen-like kringle V structure, and an inactive protease region. Each kringle structure is formed by 80 to 90 amino acids held in a triple-loop tertiary structure stabilized by disulfide bridges.9 The apo(a) component of Lp(a) may promote thrombosis by interfering with plasmin-mediated fibrinolysis, whereas the LDL component may, like common LDL, directly promote atherosclerosis.4,10 Lp(a) plasma levels are primarily genetically determined by variation in the LPA gene. Particularly influential is the KIV type 2 (KIV-2) repeat genotype of the LPA gene, determining the number of KIV protein structures and thus, the size of the expressed apo(a) protein, the size of which correlates inversely with plasma Lp(a) levels.11

Applying a Mendelian randomization study design, we tested whether elevated Lp(a) levels primarily promote thrombosis or atherosclerotic stenosis. A Mendelian randomization study examines the association of genotypes, affecting levels of a putative causal risk factor, with risk of disease.12–14 An association of genotypes with risk of disease indicates causality, because population distributions of risk alleles are generally unconfounded by behavioral and environmental factors, and because associations due to reverse causality are excluded. Thus, using both plasma Lp(a) levels and KIV-2 repeat genotypes (affecting plasma Lp(a) levels), we examined risk of venous thrombosis, risk of atherosclerotic stenosis...
in coronary, carotid, and femoral arteries, and finally risk of myocardial infarction; the latter as previously examined (with shorter follow-up and fewer events). We included myocardial infarction for comparison, because myocardial infarction may be viewed as combined thrombosis and atherosclerosis in coronary arteries. We examined a total of 41,231 individuals in 2 general population studies and 3 case–control studies, all from Copenhagen, Denmark. Finally, using instrumental variable analysis, we formally tested for an association of genetically elevated Lp(a) levels with increased risk of thrombosis and atherosclerotic stenosis.

Methods
We studied white individuals of Danish descent. All studies compiled with the Declaration of Helsinki and were approved by local ethics committees. Participants gave written informed consent. Full information on study populations, genotyping, and biochemical analyses is available in the Methods section in the online-only Data Supplement. An outline of study design is given in Figure I in the online-only Data Supplement.

Participants and Study Design
Venous Thrombosis: General Population Studies
First, we conducted a prospective general population study of venous thrombosis (deep vein thrombosis and pulmonary embolism) using the Copenhagen City Heart Study (CCHS) initiated in 1976. At the 1991–1994 examination of this cohort, blood samples for KIV-2 genotyping were available in 9289 participants and Lp(a) measurements in 9236 of those. We included 9190 participants without prior venous thrombosis. We censored participants at the occurrence of venous thrombosis, death, May 2009, whichever came first. Follow-up was 100% complete, that is, we did not lose track of a single individual during follow-up.

Second, we conducted a similar cross-sectional general population study, the Copenhagen General Population Study (CGPS) initiated in 2003 and still recruiting. We included 29,464 participants (recruited November 2003 through October 2006) for whom a KIV-2 genotype was available. Lp(a) was measured in 5547 participants. In the CGPS, a diagnosis of venous thrombosis was recorded from January 1976 through May 2009.

A factor V Leiden (FVL) genotype was available in 8689 CCHS participants and in 29,273 CGPS participants. This allowed inclusion of a positive control for risk of venous thrombosis.

Atherosclerotic Stenosis of Coronary, Carotid, and Femoral Arteries: Case–Control Studies
First, we conducted a case–control study of coronary atherosclerotic stenosis including 689 patients from the Copenhagen Ischemic Heart Disease Study. All patients had an available KIV-2 genotype and Lp(a) measurement, as well as angiographically documented coronary artery atherosclerotic disease defined as >50% stenosis (relative to the prestenotic lumen diameter) of the left main stem, or likewise >70% stenosis of another coronary artery. Patients were age- and sex-matched 1:2 to general population controls without ischemic heart disease from the CCHS.

Second, we conducted a case–control study of carotid atherosclerotic stenosis including 806 patients from the Copenhagen Carotid Stroke Study. All patients had ultrasonographically documented ≥50% carotid artery stenosis and an available KIV-2 genotype. Lp(a) measurements were available in 272 patients. Patients were age- and sex-matched 1:2 to general population controls without ischemic heart and cerebrovascular disease from the CCHS.

Third, we conducted a case–control study of peripheral artery disease affecting the lower extremities, henceforth referred to as femoral atherosclerotic stenosis, and defined as a reduced ankle-brachial index (ABI). We included participants from the CCHS with an available ABI, as determined at the 2001–2003 examination of the CCHS cohort by dividing each participant’s lowest ankle systolic blood pressure by the brachial systolic blood pressure. An ABI was available in 5274 participants who also had an Lp(a) measurement from the 2001–2003 examination, as well as an available KIV-2 genotype. An ABI ≤ 0.9 was compared with normal values of 1.11 to 1.4.21

Combined Thrombosis and Atherosclerosis in Coronary Arteries: General Population Studies
As for venous thrombosis, we conducted a prospective and a cross-sectional general population study of myocardial infarction (ie, combined thrombosis and atherosclerosis of coronary arteries), using the prospective CCHS and the cross-sectional CGPS. We included 9005 participants from the 1991–1994 examination of the CCHS cohort without prior myocardial infarction. In the CCHS, participants were censored at the occurrence of myocardial infarction, death, May 2009, whichever came first. In the CGPS, a diagnosis of myocardial infarction was recorded from January 1976 through May 2009.

Laboratory Analysis
Lp(a) was measured using a well-validated in-house turbidimetric assay or using a sensitive immunoturbidimetric assay from DiaSys.2,6
The LPA KIV-2 repeat polymorphism was genotyped by real-time polymerase chain reaction analysis yielding an estimate of the total number (sum of repeats on both alleles) of KIV-2 repeats in the LPA gene.6 FVL rs6025 was in the CCHS genotyped by polymerase chain reaction followed by restriction enzyme digest and electrophoresis,25 and in the CGPS by TaqMan analysis.

Statistical Analysis
Stata version 10.1 was used. Two-sided P < 0.05 was significant. One-way ANOVA was used to estimate the contribution of the KIV-2 genotype to the variation in plasma Lp(a) levels. Before this analysis, Lp(a) levels were square root transformed, as done by others,26 due to skewness of the distribution. For further analyses, participants were divided into groups based on tertiles of Lp(a) or tertiles of LPA KIV-2 repeats to obtain maximal statistical power. Tertiles (and FVL genotypes) were labeled 1, 2, and 3 for trend tests. In post hoc analyses of risk of venous thrombosis as a function of extreme levels of Lp(a), participants were divided into groups based on percentiles of the distribution, as done previously.26

For prospective studies, we used Cox regression analyses to estimate hazard ratios (HRs) with 95% CIs. We analyzed age-at-event using left truncation (delayed entry), and age as time scale. Analyses were age- and sex-adjusted or multivariable additionally adjusted for body mass index, smoking, leisure time physical activity, menopausal status (women only), hormone replacement therapy (women only), and oral contraceptives (women only) for venous thrombosis, and LDL cholesterol, high-density lipoprotein cholesterol, triglycerides, body mass index, hypertension, diabetes mellitus, smoking, use of lipid-lowering therapy, menopausal status, and hormone replacement therapy for myocardial infarction. Covariates relevant for women only were included in models as 3-level variables (women were coded 0 or 1, men were coded 2). LDL cholesterol levels were adjusted for the Lp(a) contribution.26 Covariates selected were classical risk factors for the relevant end point or factors known to affect levels of Lp(a). Data from the 1991–1994 and 2001–2003 examinations of the prospective CCHS were used as time-dependent covariates for multifactorial adjustments. Information on covariates was 99% complete; the few individuals lacking information were excluded from multifactorially adjusted analyses. Proportionality of hazards over time was assessed by plotting ln(−ln(survival)) versus analysis time. Suspension of nonparallel lines was further tested using Schoenfeld residuals. No violations of the proportional hazards assumption were detected. Based on the second Lp(a) measurement in 2001–2003, HRs for increased Lp(a) levels were corrected for regression dilution bias using a non-parametric method.27

Interaction of Lp(a) levels or LPA KIV-2 genotype with other covariates was evaluated by comparing models with and without 2-factor interaction terms using maximum likelihood ratio tests. No significant interactions were observed. Because no
interaction with sex was observed and because previous Lp(a) studies in the CCHS have demonstrated no sex differences, analyses were conducted nonstratified by sex to maximize statistical power.

For unmatched case–control and cross-sectional/prospective studies we used logistic regression, and for matched case–control studies conditional logistic regression analyses to estimate odds ratios (ORs) with 95% CI. Analyses were age- and sex-adjusted. Adjustment for covariates potentially affected by case status was avoided. To avoid nonlinearity in the logit, age was categorized into 8 groups in logistic regression analyses.

We used instrumental variable analysis, comparing the third and first tertile of LPA KIV-2 repeats, to calculate an HR or OR for the relevant end point for a doubling of Lp(a) levels6–33; we divided the gene end point log HR or OR (comparing the third and first KIV-2 tertile) by the mean difference in log (Lp(a)) between the third and first KIV-2 tertiles. A CI for this ratio was derived using the Fieller method. The instrumental variable ratio estimate and CI were converted to the HR or OR for the end point for a doubling of Lp(a) by converting from base $e$ to base 2 and exponentiating.

To assess the dominant pathophysiological method of action of Lp(a), we tested only 3 main prespecified hypotheses; do genetically elevated lipoprotein levels associate with increased risk of (1) venous thrombosis, (2) atherosclerotic stenosis, and (3) combined thrombosis and atherosclerosis, indicating causality? To assess causality in accordance with the principles of Mendelian randomization studies, we consequently tested for association of Lp(a) levels with risk of disease, association of LPA genotypes with Lp(a) levels, and association of LPA genotypes with risk of disease. Although many $P$ values are reported, we did not adjust for multiple testing because only 3 main and prespecified hypotheses were tested.

## Results

Basic characteristics of the white participants of Danish descent in the 2 general population studies and 3 case–control studies are shown in the Table. The subgroup of CGPS participants with an Lp(a) measurement (n=5547) had a mean age of 57 years (SD 13), and 53% were women. Baseline clinical characteristics by LPA KIV-2 genotype for all participants in the prospective CCHS are shown in Table I in the online-only Data Supplement. Median plasma Lp(a) levels were 38 mg/dL (interquartile range 12–74 mg/dL) in the first tertile of LPA KIV-2 genotype, 16 mg/dL6–33 in the second tertile, and 11 mg/dL6–22 in the third tertile (trend: $P=0.001$; Figure 1). The LPA KIV-2 polymorphism explained 22% of the total variation in plasma Lp(a) levels in the prospective CCHS and 27% in the cross-sectional CGPS.

### Venous Thrombosis: General Population Studies

Lp(a) tertiles did not associate with risk of venous thrombosis in either general population study (Figure 2). For example, in the prospective CCHS, age- and sex-adjusted HRs were 1.0 (0.8–1.4) and 0.9 (0.7–1.2) for second and third Lp(a) tertile individuals versus first tertile individuals (trend: $P=0.35$). Corresponding multifactorially adjusted HRs were 1.1 (0.8–1.4) and 0.8 (0.6–1.1) (trend: $P=0.16$).

LPA KIV-2 repeat tertiles likewise did not associate with risk of venous thrombosis in either general population study (Figure 2). For example, in the prospective CCHS, age- and sex-adjusted HRs were 1.1 (0.9–1.4) and 1.0 (0.8–1.3) for second and first KIV-2 repeat tertile individuals versus third tertile individuals (trend: $P=0.76$). Corresponding multifactorially adjusted HRs were 1.1 (0.9–1.4) and 1.0 (0.8–1.3) (trend: $P=0.95$).

In contrast, FVL genotype associated with risk of venous thrombosis in both general population studies (trend: $P<0.001$ both; Figure 2).

In post hoc analyses, combining the CCHS and the CGPS, Lp(a) tertiles did not associate with increased risk of venous thrombosis regardless of whether cases were identified prospectively or retrospectively (Figure II in the online-only Data Supplement). However, post hoc analyses of combined studies of extreme Lp(a) levels and KIV-2 genotypes demonstrated an increased risk of venous thrombosis for Lp(a) levels >95th

![Figure 1. Median lipoprotein(a) levels in the prospective Copenhagen City Heart Study as a function of tertiles of LPA kringle IV type 2 (KIV-2) repeats. Boxes mark the interquartile range, whiskers mark the 10th and 90th percentile, and dots mark the 5th and 95th percentile. P value is for Cuzick nonparametric test for trend of lipoprotein(a) levels across LPA KIV-2 tertiles.](Image)

### Table. Basic Characteristics of White Participants of Danish Descent

<table>
<thead>
<tr>
<th></th>
<th>General Population Studies</th>
<th>Case–Control Studies</th>
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<td>Coronary Atherosclerotic Stenosis</td>
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<td></td>
<td>Atherosclerosis in Coronary Arteries</td>
<td>CCHS</td>
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<tr>
<td>Age, y</td>
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<td>59 (13)</td>
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CCHS indicates Copenhagen City Heart Study; CCSS, Copenhagen Carotid Stroke Study; CGPS, Copenhagen General Population Study; CHDS, Copenhagen Ischemic Heart Disease Study; OR, odds ratio.

Age is reported as mean (SD).
percentile and LPA KIV-2 repeats <6th percentile with ORs of 1.7 (1.2–2.3) and 1.3 (1.0–1.7; Figure III in the online-only Data Supplement).

**Atherosclerotic Stenosis in Coronary, Carotid, and Femoral Arteries: Case–Control Studies**

Lp(a) tertiles associated with risk of coronary, carotid, and femoral atherosclerotic stenosis (Figure 3). Age- and sex-adjusted ORs for coronary atherosclerotic stenosis were 2.6 (2.0–3.4) and 5.0 (3.9–6.5) for second and third Lp(a) tertile individuals versus first tertile individuals (trend: \(P<0.001\)). Corresponding ORs for carotid atherosclerotic stenosis were 1.3 (0.9–1.9) and 1.7 (1.2–2.5) (trend: \(P=0.003\)), and 1.2 (1.0–1.5) and 1.6 (1.3–2.0) for femoral atherosclerotic stenosis (trend: \(P<0.001\)).

LPA KIV-2 repeat tertiles also associated with risk of coronary, carotid, and femoral atherosclerotic stenosis (Figure 3). Age- and sex-adjusted ORs for coronary atherosclerotic stenosis were 1.2 (0.9–1.5) and 1.3 (1.0–1.6) for second and first KIV-2 repeat tertile individuals versus third tertile individuals (trend: \(P=0.04\)). Corresponding ORs for carotid atherosclerotic stenosis were 1.2 (1.0–1.5) and 1.4 (1.1–1.7) (trend: \(P=0.005\)), and 1.2 (1.0–1.5) and 1.3 (1.0–1.6) for femoral atherosclerotic stenosis (trend: \(P=0.04\)).

In post hoc analyses of risk of atherosclerotic stenosis as a function of Lp(a) tertiles, risk estimates were generally only slightly altered upon adjustment for LPA genotypes (Figure IV in the online-only Data Supplement). In post hoc combined analyses of coronary, carotid, and femoral atherosclerotic stenosis (Figure V in the online-only Data Supplement), risk estimates were similar regardless of inclusion or exclusion of cases with a history of myocardial infarction. Also in post hoc analyses, elevated levels of Lp(a) associated with both moderate and severe atherosclerotic stenosis of coronary and femoral arteries (Figure VI in the online-only Data Supplement).

**Combined Thrombosis and Atherosclerosis in Coronary Arteries: General Population Studies**

Consistent with results from previous analyses (with shorter follow-up and fewer events), both Lp(a) tertiles and LPA KIV-2 repeat tertiles associated with risk of myocardial infarction in both general population studies (Figure 4) and with similar risk estimates in the 2 studies. For example, in the prospective CCHS, age- and sex-adjusted HRs were 1.0 (0.8–1.3) and 1.4 (1.2–1.8) for second and third Lp(a) tertile individuals versus first tertile individuals (trend: \(P=0.001\)), and 1.2 (1.0–1.5) and 1.4 (1.2–1.7) for second and first KIV-2 repeat tertile individuals versus third tertile individuals (trend: \(P<0.001\)). Corresponding multifactorially adjusted HRs were 1.1 (0.8–1.4) and 1.5 (1.2–1.9) (trend: \(P=0.001\)) for Lp(a) tertiles, and 1.2 (1.0–1.5) and 1.5 (1.3–1.9) (trend: \(P<0.001\)) for KIV-2 tertiles.

In contrast, FVL genotype did not associate with risk of myocardial infarction (ie, combined thrombosis and atherosclerosis in coronary arteries) in either general population study (trend: \(P=0.18\) and 0.46; Figure 4).

In post hoc analyses, combining the CCHS and the CGPS, Lp(a) tertiles did associate with increased risk of myocardial infarction regardless of whether cases were identified prospectively or retrospectively (Figure II in the online-only Data Supplement). In post hoc analyses of risk of myocardial infarction as a function of Lp(a) tertiles, risk estimates were
only slightly attenuated upon adjustment for LPA genotypes (Figure VII in the online-only Data Supplement).

**Instrumental Variable Analysis**

For a doubling of plasma Lp(a) levels, instrumental variable estimates of a HR/OR for venous thrombosis were 1.02 (0.90–1.15) in the prospective CCHS and 1.04 (0.93–1.16) in the cross-sectional CGPS (Figure 5). Correspondingly, in case–control studies, ORs were 1.12 (1.01–1.25) for coronary atherosclerotic stenosis, 1.17 (1.05–1.32) for carotid atherosclerotic stenosis, and 1.16 (1.01–1.35) for femoral atherosclerotic stenosis. Finally, for combined thrombosis and atherosclerosis in coronary arteries (ie, myocardial infarction), the corresponding HR/OR was 1.21 (1.10–1.33) in the prospective CCHS and 1.17 (1.05–1.29) in the cross-sectional CGPS.

**Discussion**

We provide genetic evidence in support of a causal association of elevated Lp(a) levels with risk of coronary, carotid, and femoral atherosclerotic stenosis, and with risk of combined thrombosis and atherosclerosis in coronary arteries (ie, myocardial infarction), but not with risk of venous thrombosis. These findings are compatible with elevated Lp(a) levels primarily promoting atherosclerotic stenosis. The present study is the first study to directly compare risk estimates for atherosclerotic stenosis, combined thrombosis and atherosclerosis, and venous thrombosis for elevated levels of Lp(a).

**Pathophysiology of Lp(a)**

Mechanistically, it is plausible that the cholesterol-laden LDL-like Lp(a) particle may contribute directly to the development of atherosclerosis. As LDL, Lp(a) can cross the
endothelial barrier, and Lp(a) may be preferentially retained in the arterial intima by binding to the extracellular matrix via both apoB and apo(a).

12 Lp(a) has been localized to atherosclerotic plaques, but not normal arteries,8,10,11 and Lp(a) has been shown to be the primary carrier of oxidized phospholipids associated with vascular inflammation and atherosclerosis.28 Data from in vitro and animal studies have implicated Lp(a) in foam cell formation, smooth muscle cell proliferation, and plaque inflammation and instability, all key processes in atherosclerosis.8,10,12 Our data support Lp(a) as a cause of

Figure 4. Combined thrombosis and atherosclerosis in coronary arteries (ie, myocardial infarction) by tertiles of Lp(a), tertiles of KIV-2 kringle IV type 2 (KIV-2) repeats, or factor V Leiden (FVL) genotype in general population studies. CCHS indicates Copenhagen City Heart Study; CGPS, Copenhagen General Population Study; Lp(a), lipoprotein(a).
atherosclerotic stenosis, because elevated plasma Lp(a) and genetically elevated Lp(a) levels associated with increased atherosclerotic stenosis, even in those that never experienced a myocardial infarction.

In vitro and animal studies have also indicated that Lp(a) may inhibit fibrinolysis through competitive inhibition of plasminogen activation and function.8,10 Of note though, because plasminogen is generally in large molar excess of Lp(a) in humans, this competitive inhibition mechanism may not be active in vivo. Furthermore, Lp(a) has in 1 in vitro study been shown to inactivate tissue factor pathway inhibitor, a potent inhibitor of the tissue factor coagulation pathway, potentially directly promoting thrombosis.9 However, most data indicate that possible prothrombotic Lp(a) effects are mediated through inhibition of fibrinolysis, and Lp(a) may therefore be expected to increase risk of venous thrombi rich in fibrin, and perhaps to a lesser degree risk of relatively fibin-poor, platelet-rich, arterial thrombi. The present data could not support Lp(a) as a cause of thrombosis, because we found no association of elevated plasma Lp(a) or genetically elevated Lp(a) levels with risk of venous thrombosis in primary analyses. However, the present data cannot completely exclude an association with arterial thrombosis distinct from that of venous thrombosis.

Comparison With Previous Studies

No previous Mendelian randomization study has compared Lp(a) risk estimates for venous thrombosis, atherosclerotic stenosis, and combined thrombosis and atherosclerosis in coronary arteries, as the present study. However, previous conventional epidemiological studies have amply documented a robust and specific association of elevated Lp(a) levels with increased risk of arterial cardiovascular disease,1–4 and recent genetic epidemiological studies, either large single-nucleotide polymorphism association studies7,29 or our own previous Mendelian randomization study of the LPA KIV-2 genotype and myocardial infarction risk,6 have provided genetic evidence of a causal association of elevated Lp(a) levels with increased risk of coronary heart disease. These recent genetic studies extend findings from early studies demonstrating association of small apo(a) isoforms with increased risk of coronary heart disease.20–32

Our finding of an increased risk of coronary, carotid, and femoral atherosclerotic stenosis as a function of elevated Lp(a) levels is generally supported by findings from previous studies.28,30,31,33–35 Our additional finding of an increased risk of coronary, carotid, and femoral atherosclerotic stenosis as a function of KIV-2 genotypes raising Lp(a) levels and results from our instrumental variable analyses support that the observed associations are causal in nature. Also, in coronary and femoral arteries elevated Lp(a) levels associated with both moderate and severe atherosclerotic stenosis in further support of causality (post hoc analyses). Our genetic findings are consistent with results from previous studies demonstrating association of small apo(a) isoforms with increased risk of coronary atherosclerosis,36,37 and with results from a very recent study demonstrating association of LPA single-nucleotide polymorphisms, associated with elevated Lp(a) plasma levels, with increased risk of carotid artery stenosis.38

Fewer studies have explored the association of elevated Lp(a) levels with risk of venous thrombosis36–41 and with conflicting results, and no studies have explored the association of genetically elevated Lp(a) levels with risk of venous thrombosis in the general population. A recent meta-analysis of 6 case–control studies involving 1826 cases and 1074 controls found that elevated Lp(a) levels (>30 mg/dL) associated with increased risk of venous thromboembolic disease,36 whereas a large prospective study including 19,921 participants found no increased risk of venous thromboembolic disease.37,38 Results from the present study of 38,654 participants in 2 large general population studies are consistent with results from the latter study, because we did not find any association of either elevated plasma Lp(a) or genetically elevated Lp(a) through KIV-2 genotypes with risk of venous thrombosis in primary analyses. Findings for venous thrombosis were insignificant regardless of whether cases were retrospectively or prospectively identified (Figure II in the online-only Data Supplement, post hoc analyses); any trend (although not statistically significant) toward an association of elevated Lp(a) levels with risk of venous thrombosis was solely seen for retrospectively identified cases. Given our results in combination with findings from previous studies,36–38 positive findings from retrospective studies may have been biased. We found the expected association of our positive control, FVL genotype, with increased risk of venous thrombosis in both general population studies. Risk estimates for venous thrombosis for FVL carriers were less extreme than previously published,29 probably reflecting the fact that we conducted general population studies as opposed to case–control studies (most previous studies), and furthermore, that we in the prospective CCHS excluded participants with events before Lp(a) measurements, and thus included only incident venous thrombosis. Of note though, our risk estimates for FVL homozygotes had wide CIs reflecting low numbers of homozygotes overall and with events.

Study Limitations

In the present study, the case for causality is based on applying a Mendelian randomization study design. Generally, randomized intervention trial data showing that lowering levels of a putative causal risk factor lead to a reduction in risk of disease is perceived to imply causality, because the random assignment to a control or intervention group likely resulted in similar distributions of known and unknown confounders. The term Mendelian randomization refers to the random assignment of genes from parents to offspring at the time of conception.14–16 Thus, a Mendelian randomization study may be considered nature’s own randomized intervention trial profiting from distributions of alleles, affecting levels of the putative causal risk factor, that are generally independent of behavioral and environmental factors. Therefore, Mendelian randomization studies likely yield unconfounded risk associations, and furthermore exclude reverse causation-based associations, thus providing evidence in support of causal associations.14–16

According to the principles of a Mendelian randomization study, an association of elevated levels of Lp(a), as well as an association of genetic variation raising levels of Lp(a), with risk of cardiovascular disease strongly favors causality.14–16 Nonetheless, final proof of causality still requires results from
randomized clinical trials demonstrating reduced risk of cardiovascular disease when targeting elevated Lp(a) levels.

The KIV-2 polymorphism explains a substantial portion of the variation in plasma Lp(a) levels, but other genetic variation unrelated to this polymorphism also influences Lp(a) plasma levels.\textsuperscript{4,13,42} thus, the KIV-2 polymorphism is not a perfect genetic correlate of plasma Lp(a) levels. Furthermore, our genotype results reflect the sum of repeats on both alleles. Such an approach may be biased because it assumes an additive effect of both alleles and a linear relationship between allele size and Lp(a) concentration, and includes nonexpressed alleles.\textsuperscript{43} Nonetheless, in the present study, the KIV-2 polymorphism explained on average $\geq 25\%$ of the total plasma Lp(a) variation, and associated with risk of cardiovascular end points in the same manner as plasma Lp(a) levels. The generally lower risk estimates obtained for the KIV-2 polymorphism compared with those for plasma Lp(a) levels are consistent with the residual plasma Lp(a) variation not explained by the KIV-2 polymorphism. Also, adjustment for the KIV-2 polymorphism (and LPA single-nucleotide polymorphisms rs3798220 and rs1045587)\textsuperscript{2} overall only slightly attenuated risk estimates for myocardial infarction and atherosclerotic stenosis for elevated Lp(a) levels (post hoc analyses).

In the present study, we assessed the presence of stenosis of coronary, carotid, and lower limb arteries by carotid ultrasonography, coronary angiography, and ABI calculation, respectively, and considered the presence of stenosis an indicator of atherosclerosis (atherosclerotic stenosis). Thus, a limitation of the present study is that we did not directly assess atherosclerosis, which would have required artery biopsies or at least intravascular ultrasound. However, arterial stenosis primarily occurs in the presence of atherosclerosis, even though stenosis and plaque burden are not identical, due to varying degrees of compensatory arterial enlargement, and may relate differently to cardiovascular disease risk factors.\textsuperscript{44} This also applies to Lp(a) where 1 study has related elevated levels to carotid stenosis and occlusion, but not to plaque burden, as assessed by carotid ultrasonography.\textsuperscript{33} As stated above, it cannot be ruled out that Lp(a) might be related to some aspect of thrombosis (promoting arterial stenosis) other than the kind of thrombosis that occurs in the setting of stasis, ie, venous thrombosis. Thus, it is possible that Lp(a) might promote plaque rupture, affect thrombosis in the setting of high shear rates, or differentially affect thrombolyis in relation to fluid dynamic effects.

We used myocardial infarction to exemplify combined arterial thrombosis and atherosclerosis, although we did not have any direct documentation of a thrombotic event (ie, cath data). However, acute rupture of an atherosclerotic plaque with superimposed thrombosis is the accepted cause of the majority of myocardial infarctions.\textsuperscript{45,46} Also, we did not directly assess the association of Lp(a) with risk of an exclusively arterial thrombotic end point, difficult in epidemiological studies because major arterial thrombotic end points primarily occur in a setting of atherosclerosis. However, although risk factors for arterial thrombosis generally differ from those for venous thrombosis,\textsuperscript{47} increasingly it is clear that there may be considerable overlap, as demonstrated by findings of increased risk of arterial cardiovascular events in patients with venous thromboembolism.\textsuperscript{48,49} Our finding of no increased risk of venous thrombosis for elevated levels of Lp(a), and our finding of similar risk estimates for coronary, carotid, and femoral atherosclerotic stenosis combined, regardless of inclusion or exclusion of cases with a history of myocardial infarction (involving both atherosclerosis and thrombosis) from analyses (post hoc), is compatible with Lp(a) primarily as a cause of atherosclerotic stenosis rather than thrombosis. Nonetheless, there may still be an association of elevated levels of Lp(a) with increased risk of thrombosis, in the right clinical setting, for instance when acting in synergy with other prothrombotic factors, or in children, where recent meta-analyses have demonstrated associations with both venous and arterial thrombotic disease,\textsuperscript{39,40} and where small apo(a) isoforms also have been associated with increased risk of venous thrombosis.\textsuperscript{41} Also, in our study, post hoc analysis of extreme levels of Lp(a) ($\geq 95\%$ percentile) and LPA KIV-2 repeat genotypes ($\leq 6\%$ percentile) did show an increased risk of venous thrombosis. The increased risk was exclusively seen for such extreme values with risk estimates of close to 1.0 for less extreme values of both Lp(a) levels and KIV-2 genotypes. These post hoc findings need independent validation.

Prior studies have reported an association of elevated Lp(a) levels with risk of stroke outcomes.\textsuperscript{3} We did not include stroke outcomes in the present article for several reasons. First, we feared reporting a false negative finding for elevated Lp(a) levels and risk of ischemic stroke, because stroke end points in Danish registries requires extensive validation, in particular to distinguish hemorrhagic from ischemic stroke. Second, ischemic stroke is of a more mixed pathogenesis than the end points explored in the present article, eg, a considerable proportion of stroke outcomes may be ascribed to the co-occurrence of cardiac arrhythmias.\textsuperscript{51}

Although Mendelian randomization studies may provide evidence of causality, because associations of genotypes with risk of disease are generally unconfounded by conventional risk factors and cannot result from reverse causation, spurious associations may result from genetic confounding caused by linkage disequilibrium or population admixture.\textsuperscript{15,52} It is unlikely that our results were confounded by population admixture because all participants in the present study were whites of Danish descent. Also, because we genotyped directly for the number of KIV-2 repeats mainly responsible for genetically different plasma Lp(a) levels,\textsuperscript{13} our present results cannot stem from linkage disequilibrium. Of note, although the ethnic uniformity of the present study avoids issues of population admixture, it also represents a limitation of the study because our results may not necessarily apply to other ethnic groups. A limitation of our study is that for all studies, a small group of participants was not included due to lack of genotype.

Conclusions

In conclusion, the present study supports, using both conventional epidemiological and Mendelian randomization approaches, that Lp(a) promotes cardiovascular disease through atherosclerotic stenosis whereas possible prothrombotic effects appear less influential. This represents important new knowledge, imperative for defining optimal future preventive treatment strategies targeting elevated Lp(a) levels.
Acknowledgments

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Disclosures

Dr Kasnstrup reported being a consultant for Abbott. Dr Nordestgaard reported being a consultant for Karo Bio, Abbott, Sanofi-Aventis, Regeneron, and AstraZeneca, and receiving lecture honoraries from Merck, AstraZeneca, and Pfizer. The other authors have no conflicts to report.

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Genetic Evidence That Lipoprotein(a) Associates With Atherosclerotic Stenosis Rather Than Venous Thrombosis
Pia R. Kamstrup, Anne Tybjaerg--Hansen and Børge G. Nordestgaard

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SUPPLEMENT MATERIAL

Supplemental Methods

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Supplemental Figure III

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SUPPLEMENTAL METHODS

We studied white individuals of Danish descent. The studies were approved by Herlev Hospital and Danish ethical committees, and were conducted according to the Declaration of Helsinki. Participants gave written informed consent. For an outline of study design please refer to Supplemental Figure I.

Participants and study design

Venous thrombosis: general population studies

First, we conducted a prospective general population study of venous thrombosis (deep vein thrombosis and/or pulmonary embolism) using the Copenhagen City Heart Study (CCHS). The CCHS is a prospective cardiovascular study of the Copenhagen general population initiated in 1976-1978 with follow-up examinations in 1981-1983, 1991-1993, and 2001-2003. Participants were randomly selected within 5-year age and sex strata from the Copenhagen Civil Registration System to reflect the population of Copenhagen aged 20-80+ years. Blood samples for DNA analysis were collected and lipoprotein(a) measurements were performed at the 1991-1994 (16563 invited, 61% response rate) and at the 2001-2003 (12599 invited, 50% response rate) examination of the cohort. We included a total of 10272 participants from the CCHS, representing participants of Danish descent with an available KIV-2 genotype and a lipoprotein(a) measurement from the 1991-1994 examination (N=9289) and/or the 2001-2003 examination (N=983); a subset of 4632 had lipoprotein(a) measurements performed at both examinations allowing correction for regression dilution bias. For the present analysis on venous thrombosis, we included 9190 1991-1994 examination participants without prior venous thrombosis.

Examinations included a self-administered questionnaire and a physical examination. Diabetes mellitus was self-reported disease, use of insulin or oral hypoglycemic drugs, and/or a non-fasting plasma glucose >11 mmol/L. Smokers were active smokers. Leisure time physical activity was divided into four categories; <2 hours per week, 2-4 hours of light exercise per week, 2-4 hours of demanding exercise per week, or >4 hours of exercise per week. Body mass index was weight in kilograms divided by height in meters squared. Hypertension was use of anti-hypertensive medication, a brachial systolic blood pressure ≥140 mmHg, and/or a diastolic blood pressure ≥90 mmHg. We followed all individuals from 1991-1994 and censored at the occurrence of venous thrombosis, death, May 2009, whichever came first. Follow-up was
100% complete, that is, we did not loose track of a single individual during follow-up; the few individuals who emigrated during follow-up were censored on the date of emigration. The mean and maximum follow-up time were 13 years and 18 years. Information on venous thrombosis, including deep venous thrombosis (DVT; ICD-8 codes 451.00, 451.08, 451.09, 451.90, 451.92, 671.01-671.09 and ICD-10 codes I80.1, I80.2, I80.3, O22.3, O87.1) and/or pulmonary embolism (PE; ICD8 codes 450.99, 673.99 and ICD-10 codes I26.0, I26.9 and O88.2) were ascertained from the national Danish Patient Registry and the national Danish Causes of Death Registry where DVT and PE diagnoses have been verified by others; 72% of diagnoses met standard diagnostic criteria which include ultrasonography or venography for DVT and ventilation-perfusion scintigraphy or pulmonary angiography for PE. The national Danish Patient Registry and the national Danish Causes of Death Registry are public registers to which all hospitalisations and deaths in Denmark are reported.

Second, we conducted a similar cross-sectional general population study of venous thrombosis using the Copenhagen General Population Study (CGPS). The CGPS is a study of the Danish general population initiated in 2003 and still recruiting. At the time of genotyping 30048 had been included (48% response rate). Participants of Danish descent were randomly selected within 5-year age and sex strata from the Copenhagen Civil Registration System to represent the population of the greater Copenhagen area aged 20-80+ years. Data collection in this study is almost identical to that of the CCHS. We included 29464 participants (recruited November 2003 through October 2006) for whom a KIV-2 genotype was available. Of those, lipoprotein(a) was measured in 5547. A diagnosis of venous thrombosis was based on the same criteria as described above and recorded in the period January 1976 through May 2009.

A factor V Leiden genotype was available in 8689 CCHS participants and in 29273 CGPS participants. This allowed inclusion of a positive control for risk of venous thrombosis, and a negative control for combined thrombosis and atherosclerosis in coronary arteries (i.e. myocardial infarction).

Atherosclerotic stenosis in coronary, carotid, and femoral arteries: case-control studies

First, we conducted a case-control study of coronary atherosclerotic stenosis including patients from the Copenhagen Ischemic Heart Disease Study (CIHDS). The CIHDS comprises patients from the greater Copenhagen area referred for coronary angiography to Copenhagen University Hospital, during the period
1991 through 1993. We included 689 patients, representing all patients with angiographically documented atherosclerotic stenosis of coronary arteries, and with an available KIV-2 genotype. Lipoprotein(a) measurements were available on all patients. Atherosclerotic stenosis of coronary arteries was defined as >50% stenosis (relative to the pre-stenotic lumen diameter) of left main stem, or likewise >70% stenosis of other coronary arteries on coronary angiography. The coronary angiography was evaluated by two experienced interventionalists, both blinded to the patients’ KIV-2 genotype and lipoprotein(a) measurement. Moderate coronary atherosclerotic stenosis was defined as 1 or 2 vessel disease (excluding main stem disease), and severe coronary atherosclerotic stenosis was defined as 3 vessel disease or main stem disease. A diagnosis of myocardial infarction was based on the same criteria as described below for the CCHS, and recorded in the period January 1976 through May 2009. Patients were age- (5-year strata) and sex-matched randomly by computer 1:2 to general population controls without ischemic heart disease from the CCHS. LPA SNP rs3798220 and rs1045587 genotypes were available on all cases and selected controls except one individual.

Second, we conducted a case-control study of carotid atherosclerotic stenosis including patients from the Copenhagen Carotid Stroke Study (CCSS). The CCSS comprises patients from the greater Copenhagen area referred for carotid artery ultrasonography to Copenhagen University Hospital, during the period 1994 through 2009. We included 806 patients, representing all patients with a stenosis of ≥50% of the diameter of the distal internal carotid artery on the most stenotic side, and an available KIV-2 genotype. Lipoprotein(a) measurements were available on a subset of 272 patients. A diagnosis of myocardial infarction was based on the same criteria as described below for the CCHS and recorded in the period January 1976 through May 2009. Cases were age- (5-year strata) and sex-matched randomly by computer 1:2 to general population controls without ischemic heart and cerebrovascular disease from the CCHS. LPA SNP rs3798220 and rs1045587 genotypes were available on all cases and selected controls.

Third, we conducted a case-control study of peripheral artery disease affecting the lower extremities, henceforth referred to as femoral atherosclerotic stenosis, and defined as a reduced Ankle Brachial Index (ABI). We included participants from the CCHS with an available KIV-2 genotype and ABI;
at the 2001-2003 examination of the CCHS cohort, 5274 participants had, in addition to a lipoprotein(a) measurement, a systolic ankle blood pressure of the posterior tibial artery on both legs obtained by Doppler. The ABI was determined by dividing each participant's lowest ankle systolic blood pressure by their brachial systolic blood pressure. An ABI of 1.1 to 1.4 was considered normal. Femoral atherosclerotic stenosis was defined as a reduced ABI; in main analyses equal to an ABI of ≤0.9, in analyses on moderate femoral atherosclerotic stenosis equal to an ABI of 1.1 to 0.81, and in analyses on severe femoral atherosclerotic stenosis equal to an ABI of ≤0.8. A diagnosis of myocardial infarction was based on the same criteria as described below for the CCHS and recorded in the period January 1976 through May 2009. LPA SNP rs3798220 and rs1045587 genotypes were available on all.

Combined thrombosis and atherosclerosis in coronary arteries: general population studies

As for venous thrombosis, we conducted a prospective and a cross-sectional/prospective general population study of myocardial infarction (i.e. combined thrombosis and atherosclerosis in coronary arteries), using the CCHS and the CGPS. We included 9005 participants from the 1991-1994 examination of the CCHS cohort without prior myocardial infarction. Participants were censored at the occurrence of myocardial infarction, death, May 2009, or emigration, whichever came first. Information on myocardial infarction diagnoses (International Classification of Diseases, 8th edition (ICD-8) codes 410, and 10th edition (ICD-10) codes I21-I22) was collected and verified by reviewing hospital admissions and diagnoses entered in the national Danish Patient Registry, causes of death entered in the national Danish Causes of Death Registry, and medical records from hospitals and general practitioners. In the CGPS, we included all 29464 CGPS participants with an available KIV-2 genotype. A diagnosis of myocardial infarction was based on the same criteria as described above and recorded in the period January 1976 through May 2009.

Laboratory analysis

In CIHDS and CCSS patients, and in participants attending the 1991-1994 examination of the CCHS, lipoprotein(a) total mass was measured, as described previously, with an in-house turbidimetric assay using a Technicon Axon autoanalyser (Miles Inc., Diagnostics Division, Tarrytown, NY, USA), rabbit anti-human lipoprotein(a) polyclonal antibodies (Q023, DAKO A/S, Glostrup, Denmark), and a human serum
lipoprotein(a) calibrator (DAKO A/S). The polyclonal antibodies did not recognize apolipoprotein B and plasminogen, and the assay was insensitive to differences in apolipoprotein(a) isoform size. At the CCHS 2001-2003 examination and at the CGPS, lipoprotein(a) was measured immediately after sampling, using a sensitive immunoturbidimetric assay from DiaSys (DiaSys Diagnostic Systems, Holzheim, Germany) containing goat anti-human lipoprotein(a) polyclonal antibodies purified by immunoabsorption against apolipoprotein B and plasminogen. For CCHS participants with a lipoprotein(a) measurement in both 1991-1994 and 2001-2003, we observed a minimal bias between the two measurements of 1.6 mg/dL, and an $r^2$ value of 0.81 (p<0.001) on linear regression.

All genotyping was performed on QIAamp 96 DNA Blood Kit (QIAGEN, Copenhagen, Denmark) purified DNA at the Department of Clinical Biochemistry at Herlev Hospital, Copenhagen University Hospital, Denmark. The LPA KIV-2 repeat polymorphism was genotyped, as described previously, by real-time PCR analysis using the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems) and 384 well formats. The single-copy gene albumin was used to normalize for different concentrations of DNA in different samples. Reactions were performed in 10 µL final volume, using 1xTaqMan Universal PCR Master Mix (Applied Biosystems), 900 nmol/L primers, and 200 nmol/L probe. Primer sequences were as follows: KIV-2 forward 5’-ATCCAGATGCTGTGGCAGCT-3’, KIV-2 reverse 5’-GCGACGGCAGTCCCTTCT-3’, albumin forward 5’-ACACGCCTTTTGCCACAATG-3’, albumin reverse 5’-CCCTGGAATAAGCCGAGCTAA-3’. The sequence for the FAM labeled KIV-2 probe was 5’-CAACCTGACGCAATGC-3’, while the sequence for the VIC labeled albumin probe was 5’-TGGGTACCTTTATTTCCTTC-3’. All samples were run in duplicate. Genotyping resulted in an estimate of the total number (sum of repeats on both alleles) of KIV-2 repeats. We prepared re-runs twice, therefore >99.9% of all participants with available DNA were genotyped. In addition to unknown samples, each 384-well plate also included a calibrator sample and two control samples, where the number of KIV-2 repeats had been determined by Southern blot analysis. Control and calibrator samples were kindly provided by Prof. Gerd Utermann and colleagues (Institut für Medizinische Biologie und Humangenetik, Universität Innsbruck, Innsbruck, Austria). To improve precision, all 41231 samples were analyzed by the
same technician, using the same calibrator and control samples, and the same ABI PRISM 7900HT Sequence Detection System.

Genotyping for factor V Leiden (rs6025; Arg506Gln mutation in coagulation factor V) in the CCHS was performed by PCR followed by restriction enzyme digest and agarose gel electrophoresis, as described previously. Genotyping for factor V Leiden in the CGPS was performed by TaqMan SNP analysis. Genotyping for LPA SNPs rs3798220 and rs1045587 was performed by TaqMan SNP analysis. Genotype distributions did not differ from those predicted by the Hardy-Weinberg equilibrium.

Enzymatic assays were used on fresh samples to measure plasma levels of total cholesterol, triglycerides, and high-density lipoprotein (HDL) cholesterol. Low-density lipoprotein (LDL) cholesterol was calculated according to the Friedewald equation if triglycerides were <4 mmol/L (~400 mg/dL), but measured using a direct method (Thermo Fischer Scientific, Waltham, MA, USA) at higher levels.
REFERENCES


Supplemental Table I. Baseline clinical characteristics by *LPA* KIV-2 genotype in the Copenhagen City Heart Study.

<table>
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<td>(8-32 repeats)</td>
<td>(33-38 repeats)</td>
<td>(39-99 repeats)</td>
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<td>3424</td>
<td>3424</td>
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<tr>
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<td>56</td>
<td>55</td>
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<tr>
<td>Age, years</td>
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<td>57 (16)</td>
<td>56 (16)</td>
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<td>3.7 (1.2)</td>
<td>3.7 (1.2)</td>
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<td>Diabetes mellitus, %</td>
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<td>2-4 h demanding exercise per week</td>
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<td>Oral contraceptives, %*</td>
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<td>Lipoprotein(a), mg/dL</td>
<td>38(12-74)</td>
<td>16(6-33)</td>
<td>11(4-22)</td>
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</table>

Continuous variables are reported as mean (SD), except for triglycerides and lipoprotein(a) that due to skewness of the distribution are reported as median (IQR). For all covariates shown data are ≥99% complete. *Women only. Abbreviations: LDL, low density lipoprotein; HDL, high density lipoprotein.
Supplemental Figure I. Outline of study design. Abbreviations: MI, myocardial infarction; ABI, ankle brachial index.

**Copenhagen General Population Study (CGPS)**
- **2003-ongoing:** KIV-2 genotype available on 29464 participants, lipoprotein(a) measurements available on 5547 of those

**Copenhagen City Heart Study (CCHS)**
- **1991-94 examination:** KIV-2 genotype on 9289 participants, lipoprotein(a) measurements on 9236 of those
- **2001-03 examination:** lipoprotein(a) measurements on 5615 participants (1036 additional to 1991-94) and ABI assessed, KIV-2 genotype on 983 additional (to 1991-94) participants

**Copenhagen Ischemic Heart Disease Study (CIHDS)**
- **1991-1993:** patients with coronary atherosclerotic stenosis recruited, KIV-2 genotype and lipoprotein(a) measurements available on 689

**Copenhagen Carotid Stroke Study (CCSS)**
- **1994-2009:** patients with ≥50% carotid atherosclerotic stenosis recruited, KIV-2 genotype available on 806 patients and lipoprotein(a) measurements on 272 of those

**Prospective study of venous thrombosis (VT):**
- lipoprotein(a) analyses included 9138, and KIV-2 genotype analyses included 9190, participants from the 1991-94 examination free of VT at baseline.

**Cross-sectional study of venous thrombosis (VT):**
- lipoprotein(a) analyses included 5547 participants (154 with a history of VT), KIV-2 genotype analyses included all 29464 participants (926 with a history of VT)

**Prospective study of combined thrombosis and atherosclerosis in coronary arteries (i.e. MI):**
- lipoprotein(a) analyses included 5547 participants (234 with a history of MI), KIV-2 genotype analyses included all 29464 participants (1165 with a history of MI)

**Cross-sectional study of combined thrombosis and atherosclerosis in coronary arteries (i.e. MI):**
- lipoprotein(a) analyses included 8952, and KIV-2 genotype analyses included 9005, participants from the 1991-94 examination free of MI at baseline.

**Association of KIV-2 genotype with lipoprotein(a) levels assessed in all 10272 (9289+983) participants**

**Case-control study of femoral atherosclerotic stenosis:**
- lipoprotein(a) and genotype analyses included participants from the 2001-03 examination with an available ABI; N = 5274

**Case-control study of coronary atherosclerotic stenosis:**
- lipoprotein(a) and KIV-2 genotype analyses included available cases (N = 689) age- and sex-matched randomly 1:2 to controls from the CCHS without ischemic heart disease

**Case-control study of carotid atherosclerotic stenosis:**
- KIV-2 genotype analyses included available cases (N=806) age- and sex-matched randomly 1:2 to controls from the CCHS without ischemic heart disease, lipoprotein(a) analyses included the subset of 272 cases with available measurements and matched CCHS controls
Supplemental Figure II. All, retrospective, and prospective venous thrombosis and myocardial infarction by tertiles of lipoprotein(a) or tertiles of **LPA KIV-2** repeats in combined general population studies. Abbreviations: CCHS, Copenhagen City Heart Study; CGPS, Copenhagen General Population Study; CI, confidence interval.

### Veins (CCHS and CGPS combined)

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### Coronary (CCHS and CGPS combined)

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<td>3</td>
<td>4608</td>
<td>186</td>
<td></td>
<td>3</td>
</tr>
</tbody>
</table>
Supplemental Figure III. Venous thrombosis by lipoprotein(a) percentile cutpoints or by LPA KIV-2 repeat percentile cutpoints in combined general population studies. Abbreviations: CCHS, Copenhagen City Heart Study; CGPS, Copenhagen General Population Study; CI, confidence interval.

Veins (CCHS and CGPS combined)

<table>
<thead>
<tr>
<th>Lp(a) (percentile)</th>
<th>Participants (N)</th>
<th>Events (N)</th>
<th>Trend: p=0.24</th>
<th>KIV-2 (percentile)</th>
<th>Participants (N)</th>
<th>Events (N)</th>
<th>Trend: p=0.22</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;22nd</td>
<td>3269</td>
<td>145</td>
<td></td>
<td>&gt;79th</td>
<td>8524</td>
<td>310</td>
<td></td>
</tr>
<tr>
<td>22nd-66th</td>
<td>6489</td>
<td>312</td>
<td></td>
<td>35th-79th</td>
<td>17050</td>
<td>659</td>
<td></td>
</tr>
<tr>
<td>67th-89th</td>
<td>3398</td>
<td>141</td>
<td></td>
<td>12th-34th</td>
<td>8914</td>
<td>320</td>
<td></td>
</tr>
<tr>
<td>90th-95th</td>
<td>885</td>
<td>35</td>
<td></td>
<td>6th-11th</td>
<td>2325</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td>&gt;95th</td>
<td>742</td>
<td>57</td>
<td></td>
<td>&lt;6th</td>
<td>1940</td>
<td>91</td>
<td></td>
</tr>
</tbody>
</table>

Odds Ratio (95% CI)
Supplemental Figure IV. Atherosclerotic stenosis in coronary, carotid, and femoral arteries by tertiles of lipoprotein(a) unadjusted for LPA genotypes, adjusted for LPA KIV-2 repeat genotype, or adjusted for LPA KIV-2 repeat genotype and LPA SNPs rs3798220 and rs1045587. Abbreviations: CCHS, Copenhagen City Heart Study; CCSS, Copenhagen Carotid Stroke Study; CIHDS, Copenhagen Ischemic Heart Disease Study; CI, confidence interval.

### Coronary (CIHDS): case-control

<table>
<thead>
<tr>
<th>Lp(a) (mg/dL)</th>
<th>Controls (N)</th>
<th>Cases (N)</th>
<th>LPA KIV-2 adjusted</th>
<th>LPA KIV-2 and SNP adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 (1-7)</td>
<td>576</td>
<td>113</td>
<td>Trend: p&lt;0.001</td>
<td>Trend: p&lt;0.001</td>
</tr>
<tr>
<td>20 (15-27)</td>
<td>455</td>
<td>234</td>
<td></td>
<td></td>
</tr>
<tr>
<td>72 (48-119)</td>
<td>347</td>
<td>342</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Odds ratio (95% CI)

### Carotid (CCSS): case-control

<table>
<thead>
<tr>
<th>Lp(a) (mg/dL)</th>
<th>Controls (N)</th>
<th>Cases (N)</th>
<th>LPA KIV-2 adjusted</th>
<th>LPA KIV-2 and SNP adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 (1-6)</td>
<td>197</td>
<td>75</td>
<td>Trend: p=0.003</td>
<td></td>
</tr>
<tr>
<td>21 (14-28)</td>
<td>183</td>
<td>89</td>
<td></td>
<td></td>
</tr>
<tr>
<td>68 (47-110)</td>
<td>164</td>
<td>108</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Odds ratio (95% CI)

### Femoral (CCHS): case-control

<table>
<thead>
<tr>
<th>Lp(a) (mg/dL)</th>
<th>Controls (N)</th>
<th>Cases (N)</th>
<th>LPA KIV-2 adjusted</th>
<th>LPA KIV-2 and SNP adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 (5-10)</td>
<td>518</td>
<td>265</td>
<td>Trend: p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>18 (15-24)</td>
<td>455</td>
<td>317</td>
<td></td>
<td></td>
</tr>
<tr>
<td>59 (42-85)</td>
<td>417</td>
<td>359</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Odds ratio (95% CI)
Supplemental Figure V. Atherosclerotic stenosis in coronary, carotid, or femoral arteries combined, by tertiles of lipoprotein(a) or LPA KIV-2 repeats in case-control studies including or excluding cases with a history of myocardial infarction. Odds ratios were derived from age and sex adjusted logistic regression analyses where duplicate Copenhagen City Heart Study controls were excluded. Abbreviations: CI, confidence interval.

**Coronary, carotid, or femoral**

<table>
<thead>
<tr>
<th>Lp(a) (mg/dL)</th>
<th>Controls (N)</th>
<th>Cases (N)</th>
<th>Lp(a) (mg/dL)</th>
<th>Controls (N)</th>
<th>Cases (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 (2-9)</td>
<td>1061</td>
<td>453</td>
<td>6 (2-9)</td>
<td>1127</td>
<td>749</td>
</tr>
<tr>
<td>19 (15-25)</td>
<td>906</td>
<td>640</td>
<td>19 (15-25)</td>
<td>1113</td>
<td>823</td>
</tr>
<tr>
<td>66 (45-105)</td>
<td>775</td>
<td>809</td>
<td>66 (45-105)</td>
<td>1066</td>
<td>864</td>
</tr>
</tbody>
</table>

**Coronary, carotid, or femoral excl. myocardial infarction**

<table>
<thead>
<tr>
<th>Lp(a) (mg/dL)</th>
<th>Controls (N)</th>
<th>Cases (N)</th>
<th>Lp(a) (mg/dL)</th>
<th>Controls (N)</th>
<th>Cases (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 (3-9)</td>
<td>724</td>
<td>320</td>
<td>7 (3-9)</td>
<td>876</td>
<td>516</td>
</tr>
<tr>
<td>19 (15-24)</td>
<td>628</td>
<td>417</td>
<td>19 (15-24)</td>
<td>861</td>
<td>554</td>
</tr>
<tr>
<td>61 (43-96)</td>
<td>569</td>
<td>480</td>
<td>61 (43-96)</td>
<td>821</td>
<td>583</td>
</tr>
</tbody>
</table>
Supplemental Figure VI. Risk of moderate or severe coronary and femoral atherosclerotic stenosis by tertiles of lipoprotein(a) in case-control studies. For coronary atherosclerotic stenosis, moderate atherosclerotic stenosis was defined as 1 or 2 vessel disease (excluding main stem disease), while severe atherosclerotic stenosis was defined as 3 vessel disease or main stem disease. For femoral atherosclerotic stenosis, moderate atherosclerotic stenosis was defined as an ABI of 1.1 to 0.81, while severe atherosclerotic stenosis was defined as an ABI of \( \leq 0.8 \). Abbreviations: CIHDS, Copenhagen Ischemic Heart Disease Study; CCHS, Copenhagen City Heart Study; CI, confidence interval.

### Coronary (CIHDS)

<table>
<thead>
<tr>
<th>Lp(a) (mg/dL)</th>
<th>Controls (N)</th>
<th>Cases (N)</th>
<th>Odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 (1-7)</td>
<td>293</td>
<td>57</td>
<td>Trend: ( p&lt;0.001 )</td>
</tr>
<tr>
<td>19 (15-26)</td>
<td>230</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>67 (43-111)</td>
<td>177</td>
<td>173</td>
<td></td>
</tr>
</tbody>
</table>

### Severe atherosclerotic stenosis

<table>
<thead>
<tr>
<th>Lp(a) (mg/dL)</th>
<th>Controls (N)</th>
<th>Cases (N)</th>
<th>Odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 (1-6)</td>
<td>286</td>
<td>53</td>
<td>Trend: ( p&lt;0.001 )</td>
</tr>
<tr>
<td>19 (14-25)</td>
<td>239</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>72 (46-121)</td>
<td>153</td>
<td>186</td>
<td></td>
</tr>
</tbody>
</table>

### Femoral (CCHS)

<table>
<thead>
<tr>
<th>Lp(a) (mg/dL)</th>
<th>Controls (N)</th>
<th>Cases (N)</th>
<th>Odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 (5-11)</td>
<td>522</td>
<td>1099</td>
<td>Trend: ( p&lt;0.001 )</td>
</tr>
<tr>
<td>19 (15-23)</td>
<td>442</td>
<td>1151</td>
<td></td>
</tr>
<tr>
<td>55 (40-80)</td>
<td>426</td>
<td>1180</td>
<td></td>
</tr>
</tbody>
</table>

### Severe atherosclerotic stenosis

<table>
<thead>
<tr>
<th>Lp(a) (mg/dL)</th>
<th>Controls (N)</th>
<th>Cases (N)</th>
<th>Odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 (5-11)</td>
<td>513</td>
<td>101</td>
<td>Trend: ( p&lt;0.001 )</td>
</tr>
<tr>
<td>18 (15-23)</td>
<td>457</td>
<td>153</td>
<td></td>
</tr>
<tr>
<td>60 (42-87)</td>
<td>420</td>
<td>191</td>
<td></td>
</tr>
</tbody>
</table>
Supplemental Figure VII. Combined thrombosis and atherosclerosis in coronary arteries (i.e. myocardial infarction) by tertiles of lipoprotein(a) unadjusted for LPA genotypes, adjusted for LPA KIV-2 repeat genotype, or adjusted for LPA KIV-2 repeat genotype and LPA SNPs rs3798220 and rs1045587. Abbreviations: CCHS, Copenhagen City Heart Study; CGPS, Copenhagen General Population Study; CI, confidence interval.

Coronary (CCHS): prospective

<table>
<thead>
<tr>
<th>Lp(a) (mg/dL)</th>
<th>Participants (N)</th>
<th>Events (N)</th>
<th>LPA KIV-2 adjusted</th>
<th>LPA KIV-2 and SNP adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 (1-5)</td>
<td>2996</td>
<td>206</td>
<td>Trend: p=0.001</td>
<td></td>
</tr>
<tr>
<td>17 (12-23)</td>
<td>2991</td>
<td>223</td>
<td>Trend: p=0.02</td>
<td></td>
</tr>
<tr>
<td>59 (40-94)</td>
<td>2965</td>
<td>286</td>
<td>Trend: p=0.04</td>
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</tr>
</tbody>
</table>

Hazard Ratio (95% CI)

Coronary (CGPS): cross-sectional

<table>
<thead>
<tr>
<th>Lp(a) (mg/dL)</th>
<th>Controls (N)</th>
<th>Cases (N)</th>
<th>LPA KIV-2 adjusted</th>
<th>LPA KIV-2 and SNP adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td>9 (6-12)</td>
<td>1804</td>
<td>62</td>
<td>Trend: p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>20 (17-25)</td>
<td>1765</td>
<td>69</td>
<td>Trend: p=0.005</td>
<td></td>
</tr>
<tr>
<td>61 (43-87)</td>
<td>1743</td>
<td>104</td>
<td>Trend: p=0.02</td>
<td></td>
</tr>
</tbody>
</table>

Odds ratio (95% CI)