Genetically Elevated Lipoprotein(a) and Increased Risk of Myocardial Infarction

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Context High levels of lipoprotein(a) are associated with increased risk of myocardial infarction (MI).

Objective To assess whether genetic data are consistent with this association being causal.

Design, Setting, and Participants Three studies of white individuals from Copenhagen, Denmark, were used: the Copenhagen City Heart Study (CCHS), a prospective general population study with 16 years of follow-up (1991-2007, n=8637, 599 MI events); the Copenhagen General Population Study (CGPS), a cross-sectional general population study (2003-2006, n=29 388, 994 MI events); and the Copenhagen Ischemic Heart Disease Study (CIHDS), a case-control study (1991-2004, n=2461, 1231 MI events).

Main Outcome Measures Plasma lipoprotein(a) levels, lipoprotein(a) kringle IV type 2 (KIV-2) size polymorphism genotype, and MIs recorded from 1976 through July 2007 for all participants.

Results In the CCHS, multivariable-adjusted hazard ratios (HRs) for MI for elevated lipoprotein(a) levels were 1.2 (95% confidence interval [CI], 0.9-1.6; events/10 000 person-years, 59) for levels between the 22nd and 66th percentile, 1.6 (95% CI, 1.1-2.2; events/10 000 person-years, 75) for the 67th to 89th percentile, 1.9 (95% CI, 1.2-3.0; events/10 000 person-years, 84) for the 90th to 95th percentile, and 2.6 (95% CI, 1.6-4.1; events/10 000 person-years, 108) for levels greater than the 95th percentile, respectively, vs levels less than the 22nd percentile (events/10 000 person-years, 55) (trend $P<.001$). Numbers of KIV-2 repeats (sum of repeats on both alleles) ranged from 6 to 99 and on analysis of variance explained 21% and 27% of all variation in plasma lipoprotein(a) levels in the CCHS and CGPS, respectively. Mean lipoprotein(a) levels were 56, 31, 20, and 15 mg/dL for the first, second, third, and fourth quartiles of KIV-2 repeats in the CCHS, respectively (trend $P<.001$); corresponding values in the CGPS were 60, 34, 22, and 19 mg/dL (trend $P<.001$). In the CCHS, multivariable-adjusted HRs for MI were 1.5 (95% CI, 1.2-1.9; events/10 000 person-years, 75), 1.3 (95% CI, 1.0-1.6; events/10 000 person-years, 66), and 1.1 (95% CI, 0.9-1.4; events/10 000 person-years, 57) for individuals in the first, second, and third quartiles, respectively, as compared with individuals in the fourth quartile of KIV-2 repeats (events/10 000 person-years, 51) (trend $P<.001$). Corresponding odds ratios were 1.3 (95% CI, 1.1-1.5), 1.1 (95% CI, 0.9-1.3), and 0.9 (95% CI, 0.8-1.1) in the CGPS (trend $P=.005$), and 1.4 (95% CI, 1.1-1.7), 1.2 (95% CI, 1.0-1.6), and 1.3 (95% CI, 1.0-1.6) in the CIHDS (trend $P=.01$). Genetically elevated lipoprotein(a) was associated with an HR of 1.22 (95% CI, 1.09-1.37) per doubling of lipoprotein(a) level on instrumental variable analysis, while the corresponding value for plasma lipoprotein(a) levels on Cox regression was 1.08 (95% CI, 1.03-1.12).

Conclusion These data are consistent with a causal association between elevated lipoprotein(a) levels and increased risk of MI.

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MYOCARDIAL INFARCTION (MI) remains a leading cause of morbidity and mortality despite targeting of low-density lipoprotein (LDL) cholesterol by statin therapy. The need for identification of additional causal factors, and thus potential new targets for prophylactic treatment, is apparent. Elevated levels of lipoprotein(a) represent such a candidate[1-5]; however, whether lipoprotein(a) causes MI is unclear. A randomized intervention trial showing that a reduction in lipoprotein(a) levels leads to a reduction in risk of MI would favor causality. Such a study has yet to be conducted. Alternatively, a mendelian randomization study can also provide evidence of causal relationships profiting from population distributions of risk alleles that are generally independent of behavioral and environmental factors, thus yielding largely unconfounded risk associations and excluding associations caused by reverse causation.[6-8] Simply put, association of elevated levels of lipoprotein(a), as well as association of genetic variation raising levels of lipoprotein(a), with risk of MI would suggest causality.

Lipoprotein(a) consists of what is essentially an LDL particle bound to a plasminogen-like glycoprotein, apolipoprotein(a).[9] Levels of lipoprotein(a) may vary up to a thousandfold among individuals, and levels are partly determined by polymorphisms in the *LPA* gene (OMIM 152200) coding for the apolipoprotein(a) moiety of

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lipoprotein(a). The most influential *LPA* polymorphism is the kringle IV type 2 (KIV-2) size polymorphism, a copy number variant defined by a variable number of 5.6-kilobase (kb) repeats and resulting in a large number of differently sized isoforms of apolipoprotein (a). The number of KIV-2 repeats correlates inversely with levels of lipoprotein(a).[9]

We examined the hypothesis that genetically elevated lipoprotein(a) levels cause increased risk of MI. We tested first whether elevated plasma lipoprotein(a) levels are associated with increased risk of MI; second, whether lipoprotein(a) KIV-2 size polymorphism genotype is associated with increased lipoprotein(a) levels; and third, whether lipoprotein(a) KIV-2 size polymorphism genotype is associated with an increased risk of MI that is consistent with its effect on lipoprotein(a) levels.

METHODS

Participants

We studied 3 cohorts of white individuals of Danish descent. These groups were defined so that no individual appears twice in any of the 3 analysis groups, thus permitting independent confirmation of findings in each group. The studies were approved by Herlev Hospital and Danish ethical committees (KF100.2039/91, H-KF01-144/01, KA93125, and KA99039) and were conducted according to the Declaration of Helsinki. Participants gave written informed consent.

The Copenhagen City Heart Study (CCHS) is a prospective cardiovascular study of the Copenhagen general population initiated in 1976.[10] Participants were selected from the Copenhagen Civil Registration System to represent the population of Copenhagen aged 20 years and older. We included a total of 9867 participants; 8751 from the 1991-1994 examination of the cohort and an additional 1116 from the 2001-2003 examination. At the examinations, blood samples for DNA analysis were collected and lipoprotein(a) measurements performed. Of the 9867 participants, 4514 had lipoprotein(a) measurements performed at both examinations, allowing correction for regression dilution bias.[11] All participants included in the present study were initially free of ischemic heart disease. Of the 9867 participants from the CCHS, 1230 of those who remained free of MI at the end of follow-up in 2007 were randomly sampled by a computer (within 5-year age and sex strata) to function as controls for the 1231 patients with a history of MI in the Copenhagen Ischemic Heart Disease Study (CIHDS). The remaining 8637 were analyzed as a single cohort for analysis of the association of lipoprotein(a) plasma levels or KIV-2 genotype with risk of MI.

Examinations included a self-administered questionnaire and a physical examination. Hypertension was defined as use of antihypertensive medication, a systolic blood pressure of 140 mm Hg or greater, or a diastolic blood pressure of 90 mm Hg or greater. Diabetes mellitus was defined as self-reported disease, use of insulin or oral hypoglycemic drugs, or nonfasting plasma glucose greater than 11 mmol/L. Smokers were active smokers. Body mass index was calculated as weight in kilograms divided by height in meters squared.

We followed up all individuals from January 1976 until the occurrence of MI, death, or July 2007, whichever came first. Follow-up was 100% complete; that is, we did not lose any individuals during follow-up. Information on diagnosis of MI (World Health Organization; code 410 from the *International Classification of Diseases, Eighth Revision* [*ICD-8*] and codes I21-I22 from the *ICD-10*) 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. All hospitals in Denmark report to the Danish Patient Registry, and all death certificates in Denmark are registered in the Danish Causes of Death Registry. Autopsies are not performed routinely. Information from the registries was gathered at regular intervals of approximately 3 years. A diagnosis of MI was confirmed from discharge records by cardiologists until 1994. Because a sample of 200 cases of MI then showed that at least 99.5% were correctly diagnosed in the national Danish Patient Registry, further individual confirmation of MI diagnoses was no longer performed. A diagnosis of MI required the presence of at least 2 of the following criteria: characteristic chest pain, elevated cardiac enzymes, or electrocardiographic changes indicative of MI.

The Copenhagen General Population Study (CGPS) is a cross-sectional study of the Danish general population initiated in 2003 and still recruiting.[12,13] Participants were selected from the Copenhagen Civil Registration System to represent the population of Copenhagen aged 20 years and older. Data collection in this study was almost identical to that of the CCHS. We included 29 388 participants for whom a KIV-2 genotype was available. Of those, lipoprotein(a) was measured in 5543. A diagnosis of MI was based on the same criteria as described earlier and recorded in the period January 1976 through July 2007. Of the 29 388 participants, 994 were diagnosed with an MI.

The CIHDS comprises patients from the greater Copenhagen area referred for coronary angiography to Copenhagen University Hospital during the period 1991 through 2004. All 1231 patients included in the present study had a history of MI as well as an available KIV-2 genotype. A diagnosis of MI was based on the same criteria described here and recorded in the period January 1976 through July 2007. Cases were age (5-year strata) and sex matched to 1230 controls without ischemic heart disease from the CCHS.

Laboratory Analysis

The lipoprotein(a) KIV-2 size polymorphism was genotyped by real-time polymerase chain reaction (PCR) analysis using the ABI Prism 7900HT Sequence Detection System (Applied Biosystems; Foster City, California) and 384 well for-

mats (eAppendix available at http://www.jama.com). This assay was developed specifically for the present study. 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, more than 99.9% of all participants with available DNA were genotyped.

In participants attending the 1991-1994 examination of the CCHS, lipoprotein(a) total mass was measured using an in-house method, as described previously[3] (eAppendix). At the 2001-2003 examination of the CCHS and for CGPS participants, lipoprotein(a) was measured immediately after sampling using a sensitive immunoturbidimetric assay from DiaSys (DiaSys Diagnostic Systems, Holzheim, Germany). Enzymatic assays were used on fresh samples to measure plasma levels of total cholesterol and triglycerides.

Statistical Analysis

Stata statistical software package version 9.2 was used for analysis (StataCorp, College Station, Texas). A 2-sided $P < .05$ was considered significant. In the CCHS and CGPS, 1-way analysis of variance (ANOVA) was used to estimate the contribution of the KIV-2 genotype to the variation in plasma lipoprotein(a) levels. Prior to this analysis, lipoprotein(a) levels were square-root transformed, as done by others,[14] because of the skewness of the distribution. For further analyses of the 3 studies, participants were divided into groups based on quartiles of total number of KIV-2 repeats, as specified a priori.

Additionally, we examined risk of MI according to total number of KIV-2 repeats on a continuous scale. In the prospective CCHS with 8637 participants and 599 MI events, we had 90% statistical power at a 2-sided $P < .05$ to detect a hazard ratio (HR) of 1.18 per decrease of 10 KIV-2 repeats. To illustrate the robustness of the found association of KIV-2 genotype with lipoprotein(a) levels, results for octiles of total number of KIV-2 repeats are also displayed for the CCHS. Cuzick nonparametric test for trend was used to test for differences in lipoprotein(a) levels between different KIV-2 genotype quantiles.

In the CCHS, we used Cox regression to analyze age-at-event using left truncation (delayed entry) and age as time scale. Thus, age was automatically adjusted for, and we took into account that a period of unawareness exists before an individual enters the study, a period in which the individual may have been subjected to the effects of elevated lipoprotein(a) levels. Only participants not selected as controls for the CIHDS were included. We examined risk of MI as a function of KIV-2 genotype quartiles or baseline plasma lipoprotein(a) levels at the third examination in 1991-1994. Lipoprotein(a) cut points were defined a priori based on percentiles of the distribution, as done previously.[3]

Analyses were age and sex adjusted or multivariable adjusted for age, sex, total cholesterol (corrected for the lipoprotein[a] contribution), triglycerides, body mass index, hypertension, diabetes mellitus, smoking, and use of lipid-lowering therapy and in women also for menopausal status and use of hormone therapy. Data from the 1991-1994 and 2001-2003 examinations were used as time-dependent covariates for multivariable adjustments. Therefore, covariate values changed over the years of follow-up dependent on availability of data from the 2001-2003 examination. Total cholesterol level, dependent on an available lipoprotein(a) measurement from the same examination, was adjusted for the lipoprotein(a) contribution, assuming a 15% contribution of cholesterol to total lipoprotein(a) mass.[15] Based on the second lipoprotein(a) measurement in 2001-2003, HRs for increased lipoprotein(a) levels were corrected for regression dilution bias using a nonparametric method.[11]

Interaction of KIV-2 genotype or lipoprotein(a) levels with other covariates was evaluated by comparing models with and without interaction terms using maximum likelihood ratio tests. With age as time scale, we could not study the effects of age itself. Therefore, for the test of interaction of age with KIV-2 genotype or lipoprotein(a) levels, we used years of follow-up as the time scale analyzing time to event. Proportionality of hazards over time was assessed by plotting ln(–ln[survival]) vs analysis time for models including quantiles of KIV genotype or lipoprotein(a) levels and other covariates (age and sex or multivariable adjusted). No violations of the proportional hazards assumption were detected. Evidence for nonlinear trend of increases in risk of MI for increasing levels of lipoprotein(a), or for decreasing numbers of KIV-2 repeats, was tested for using a likelihood ratio test comparing models including lipoprotein(a) levels or KIV-2 repeats on a continuous scale to models including quantiles of lipoprotein(a) or KIV-2 repeats. We found no evidence of nonlinearity (both P values $> .99$).

In the CGPS, we used logistic regression, and in the matched CIHDS, conditional logistic regression, to estimate odds ratios (ORs) with 95% confidence intervals (CIs). Analyses were age and sex adjusted or multivariable adjusted for age, sex, and diabetes mellitus. Adjustment for covariates potentially affected by case status was avoided. Continuous covariates were tested for linearity in the logit using a Box-Tidwell transformation; in the CGPS, age was logarithmically transformed to avoid nonlinearity in the logit.

In the CCHS with complete information on both KIV-2 genotype and lipoprotein(a) levels for all participants, we performed instrumental variable analysis[16] to further assess evidence of causality. We calculated the instrumental variable estimate of the log HR of MI per unit increase in log (lipoprotein[a]) using a ratio estimate: we divided the gene-MI log HR (comparing the fourth and first KIV-2 quartiles) by the mean difference in log (lipoprotein[a]) between the fourth and first KIV-2 quartiles. A CI for the ratio was estimated using Fieller method. The estimate and CI for the instrumental vari-

able ratio was converted to the HR of MI for a doubling of lipoprotein(a) by multiplying by 2/*e* and exponentiating.

Only 3 main, prespecified hypotheses (in accordance with the principles of mendelian randomization studies) were tested: whether levels of lipoprotein(a) are associated with risk of MI, whether KIV-2 genotype is associated with levels of lipoprotein(a), and whether KIV-2 genotype is associated with risk of MI. Therefore, although many *P* values are reported, we did not adjust for multiple comparison.

Table 1. Basic Characteristics of Participants (White Individuals of Danish Descent) in the 3 Studies

	CCHS	CGPS	CIHDS
Total, No.	8637	29 388	2461
Women, No. (%)	5302 (61)	15 260 (52)	566 (23)
Age, mean (SD), y	55 (17)	59 (13)	60 (10)
Diabetes mellitus, No. (%)	311 (4)	1346 (5)	242 (10)

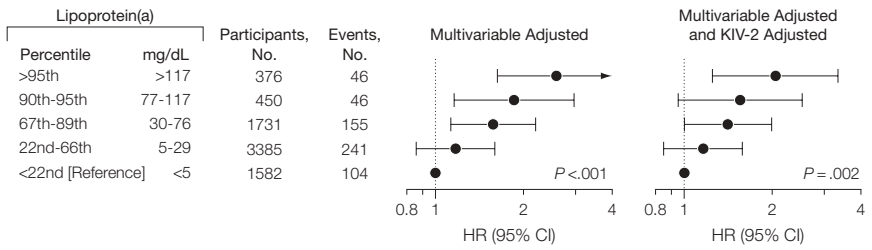
Abbreviations: CCHS, Copenhagen City Heart Study; CGPS, Copenhagen General Population Study; CIHDS, Copenhagen Ischemic Heart Disease Study.

Table 2. Baseline Cardiovascular Risk Factors by Genotype for Participants in the Copenhagen City Heart Study[a]

	Kringle IV Type 2 Quartile			
	First (6-30 Repeats)	Second (31-35 Repeats)	Third (36-40 Repeats)	Fourth (41-99 Repeats)
Total, No.	2467	2467	2467	2466
Women, No. (%)	1378 (56)	1401 (57)	1442 (58)	1364 (55)
Age, mean (SD), y	55 (16)	56 (16)	56 (16)	55 (16)
Total cholesterol, mean (SD), mg/dL	236 (50)	232 (50)	232 (50)	232 (50)
Triglycerides, median (IQR), mg/dL	134 (89-187)	134 (89-196)	134 (98-196)	134 (98-196)
Body mass index, mean (SD)[b]	25.5 (4.3)	25.4 (4.2)	25.6 (4.4)	25.5 (4.4)
Hypertension, No. (%)	1226 (50)	1279 (52)	1241 (51)	1243 (51)
Smoking, No. (%)	1127 (46)	1171 (48)	1183 (48)	1174 (48)
Diabetes mellitus, No. (%)	94 (4)	93 (4)	85 (3)	102 (4)
Lipid-lowering therapy, No. (%)	20 (0.8)	15 (0.6)	16 (0.7)	17 (0.7)
Postmenopausal status, No. (%)[c]	900 (66)	942 (68)	973 (68)	897 (66)
Hormone therapy, No. (%)[d]	172 (19)	203 (22)	183 (19)	172 (19)

Abbreviation: IQR, interquartile range.
SI conversion factors: To convert total cholesterol to mmol/L, multiply by 0.0259; to convert triglycerides to mmol/L, multiply by 0.0113.
[a] All participants were white individuals of Danish descent. Numbers include participants used as controls in the Copenhagen Ischemic Heart Disease Study.
[b] Calculated as weight in kilograms divided by height in meters squared.
[c] Among women only.
[d] Among postmenopausal women only.

Figure 1. Risk of Myocardial Infarction by Extreme Levels of Lipoprotein(a) in the General Population



Hazard ratios (HRs) were multivariable adjusted for age, sex, total cholesterol (corrected for the lipoprotein[a] contribution), triglycerides, body mass index, hypertension, diabetes mellitus, smoking, and use of lipid-lowering therapy and for women also for menopause and hormone therapy or for all of these variables as well as kringle IV type 2 (KIV-2) genotype. *P* values are test for trend of hazard ratios where lipoprotein(a) groups with increasing levels were coded 1, 2, 3, 4, and 5. Values are from the 1991-1994 examination of the Copenhagen City Heart Study with up to 16 years of follow-up (n=7524). Controls used in the Copenhagen Ischemic Heart Disease Study (n=1200) were excluded from analysis. CI indicates confidence interval.

RESULTS

Basic characteristics of the white participants of Danish descent in the 3 studies from Copenhagen, Denmark, are shown in TABLE 1. Among participants in the CCHS, cardiovascular risk factors did not differ by KIV-2 genotype (TABLE 2).

Plasma Lipoprotein(a) Levels and MI

In accordance with previous findings,[3,17] elevated lipoprotein(a) levels were associated with increased risk of MI (FIGURE 1) (trend, $P<.001$), with multivariable-adjusted HRs of 1.2 (95% CI, 0.9-1.6; events/10 000 person-years, 59) for levels between the 22nd and 66th percentile, 1.6 (95% CI, 1.1-2.2; events/10 000 person-years, 75) for levels between the 67th and 89th percentile, 1.9 (95% CI, 1.2-3.0; events/10 000 person-years, 84) for levels between the 90th and 95th percentile, and 2.6 (95% CI, 1.6-4.1; events/10 000 person-years, 108) for levels greater than the 95th percentile, respectively, vs levels less than the 22nd percentile (events/10 000 person-years, 55). On additional adjustment for the KIV-2 genotype, HRs were attenuated (Figure 1). No significant interactions were observed between lipoprotein(a) levels and age, sex, total cholesterol (corrected for the lipoprotein[a] contribution), triglycerides, body mass index, hypertension, diabetes mellitus, smoking, lipid-lowering therapy, menopause, or use of hormone therapy on risk of MI in the CCHS (*P* values of .16 to .97 for tests of interaction).

KIV-2 Size Polymorphism and Plasma Lipoprotein(a) Levels

Genotyping revealed a range in total number of KIV-2 repeats (sum of repeats on both alleles) from 6 to 99. The number of repeats was inversely associated with lipoprotein(a) levels (FIGURE 2) (all trend $P<.001$), and the KIV-2 size polymorphism explained 21% of all variation in lipoprotein(a) levels in the CCHS

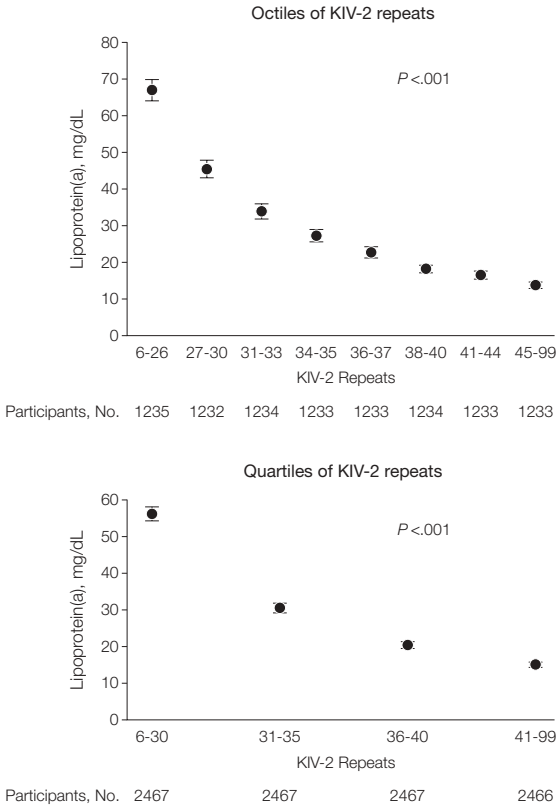
(ANOVA: F_{65}=41) and 27% in the CGPS (ANOVA: F_{54}=39). Mean lipoprotein(a) levels were 56, 31, 20, and 15 mg/dL for the first, second, third, and fourth quartiles of KIV-2 repeats, respectively, in the CCHS (Figure 2); corresponding values for the CGPS were 60, 34, 22, and 19 mg/dL.

When stratifying participants in the 1991-1994 examination of the CCHS into 4 groups based on quartiles of age at the time of examination, the stepwise decrease in mean lipoprotein(a) levels with increasing KIV-2 repeats was seen for all age groups (TABLE 3) (all trend $P<.001$, post hoc analysis). Likewise, when considering participants who attended the 1991-1994 as well as the 2001-2003 examination of the CCHS, the same inverse association of number of KIV-2 repeats with levels of lipoprotein(a) was seen at both examinations (both trend $P<.001$, post hoc analysis).

KIV-2 Size Polymorphism and MI

A low number of KIV-2 repeats was associated with increased risk of MI in all 3 studies (FIGURE 3) (tests for trend ranged from $P=.03$ to $P<.001$). Multivariable-adjusted HRs for MI in the CCHS were 1.5 (95% CI, 1.2-1.9; events/10 000 person-years, 75), 1.3 (95% CI, 1.0-1.6; events/10 000 person-years, 66), and 1.1 (95% CI, 0.9-1.4; events/10 000 person-years, 57) for individuals in the first, second, and third quartiles, respectively, as compared with individuals in the fourth quartile of KIV-2 repeats (events/10 000 person-years, 51). Corresponding ORs in the CGPS were 1.3 (95% CI, 1.1-1.5), 1.1 (95% CI, 0.9-1.3), and 0.9 (95% CI, 0.8-1.1), and in the CIHDS, 1.4 (95% CI, 1.1-1.7), 1.2 (95% CI, 1.0-1.6), and 1.3 (95% CI, 1.0-1.6). On a continuous scale, a decrease in 10 KIV-2 repeats was associated with a multivariable-adjusted HR for MI of 1.3 (95% CI, 1.1-1.4) in the CCHS, with corresponding ORs of 1.1 (95% CI, 1.0-1.2) in the CGPS and 1.1 (95% CI, 1.0-1.3) in the CIHDS. On additional adjustment for plasma lipoprotein(a) levels in the CCHS, a decrease in 10 KIV-2 repeats was associated with an HR for MI of 1.2 (95% CI, 1.0-1.3). No significant interactions were observed between KIV-2 genotype

Figure 2. Mean Lipoprotein(a) Levels in the CCHS as a Function of Octiles or Quartiles of Apolipoprotein(a) KIV-2 Repeats



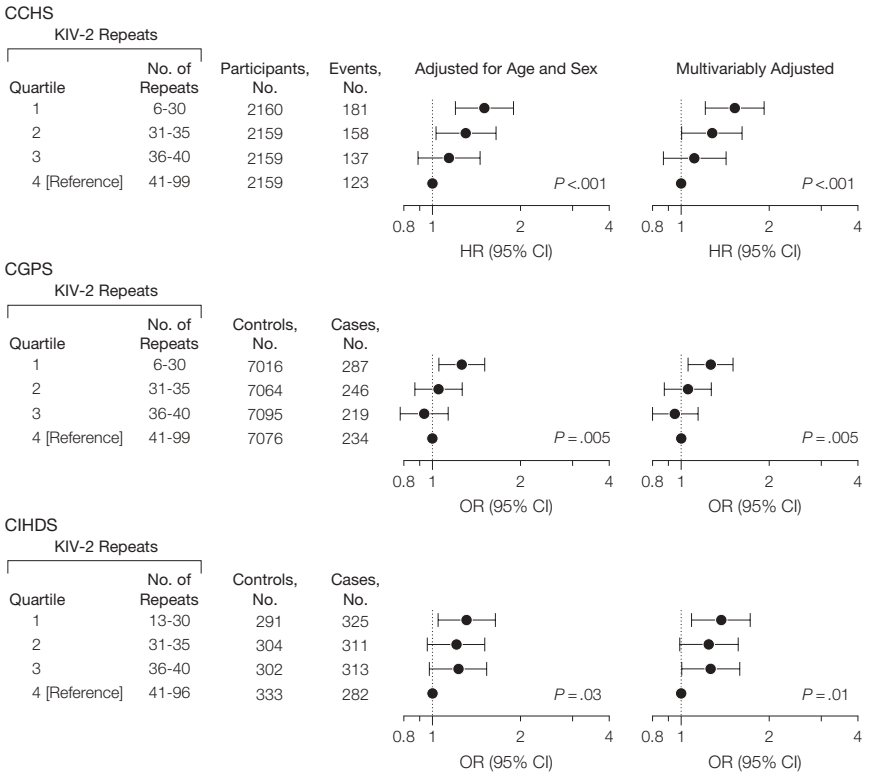
P values are for Cuzick nonparametric test for trend of mean lipoprotein(a) levels. Participants in the 1991-1994 or 2001-2003 examination were included (n=9867). CCHS indicates Copenhagen City Heart Study; KIV-2, kringle IV type 2. Error bars indicate 95% confidence intervals.

Table 3. Lipoprotein(a) Levels as a Function of Kringle IV Type 2 Repeats in the Copenhagen City Heart Study[a]

	Lipoprotein(a), Mean (95% CI), mg/dL			
	First Quartile (6-30 KIV-2 Repeats)	Second Quartile (31-35 KIV-2 Repeats)	Third Quartile (36-40 KIV-2 Repeats)	Fourth Quartile (41-99 KIV-2 Repeats)
Participants in the 1991-1994 examination by age, y				
21-46 (n = 2204)	52 (48-55)	27 (25-30)	16 (14-18)	12 (11-13)
47-59 (n = 2233)	55 (51-59)	31 (28-35)	21 (19-23)	14 (13-16)
60-69 (n = 2177)	61 (57-66)	30 (28-33)	22 (20-24)	15 (14-17)
70-93 (n = 2137)	61 (56-65)	34 (31-37)	21 (20-23)	18 (16-20)
Participants who attended both examinations (n = 4514)				
1991-1994 Examination	57 (54-59)	31 (29-33)	20 (19-22)	15 (14-16)
2001-2003 Examination	58 (55-60)	32 (30-34)	22 (21-23)	17 (16-18)

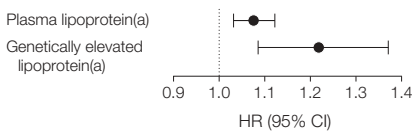
Abbreviations: CI, confidence interval; KIV-2, kringle IV type 2.
[a] $P<.001$ in all tests for trend of lipoprotein(a) levels by KIV-2 genotype quartile.

Figure 3. Risk of Myocardial Infarction by Quartiles of Apolipoprotein(a) KIV-2 Repeats in the CCHS, CGPS, and CIHDS



In the Copenhagen City Heart Study (CCHS), risk estimates were adjusted for age and sex or multivariably for age, sex, total cholesterol (corrected for the lipoprotein[a] contribution), triglycerides, body mass index, hypertension, diabetes mellitus, smoking, and use of lipid-lowering therapy and for women also for menopause and hormone therapy. In the Copenhagen General Population Study (CGPS) and Copenhagen Ischemic Heart Disease Study (CIHDS), odds ratios (ORs) were adjusted for age and sex or multivariably for age, sex, and diabetes mellitus. *P* values are test for trend of risk estimates (hazard ratios [HRs] or ORs) where kringle IV type 2 (KIV-2) groups with decreasing numbers of KIV-2 repeats were coded 1, 2, 3, and 4. There was no overlap of individuals between studies. Participants in the CGPS with incomplete information on covariates were dropped from analysis (n=151); otherwise, numbers of individuals included are as shown in Table 1. CI indicates confidence interval (shown as error bars).

Figure 4. Risk of Myocardial Infarction for Doubling in Lipoprotein(a) Levels in the CCHS



The risk estimate for a doubling in plasma lipoprotein(a) was calculated using Cox regression while that for genetically elevated lipoprotein(a) was derived from an instrumental variable analysis. Hazard ratios (HRs) were multifactorially adjusted for age, sex, total cholesterol (corrected for the lipoprotein[a] contribution), triglycerides, body mass index, hypertension, diabetes mellitus, smoking, and use of lipid-lowering therapy and for women also for menopause and hormone therapy. CCHS indicates Copenhagen City Heart Study; CI, confidence interval.

and age, sex, total cholesterol (corrected for the lipoprotein[a] contribution), triglycerides, body mass index, hypertension, diabetes mellitus, smoking, lipid-lowering therapy, menopause, or use of hormone therapy on risk of MI in the CCHS (*P* values of .05 to .86 for tests of interaction).

Plasma Levels vs Genetically Elevated Levels of Lipoprotein(a) on Risk of MI

A doubling of plasma lipoprotein(a) levels yielded a multivariable-adjusted HR for MI of 1.08 (95% CI, 1.03-1.12) on Cox regression in the CCHS (FIGURE 4). Combining the estimate of the difference in lipoprotein(a) levels between the first and fourth KIV-2 quartiles (Figure 2) with the multivariable-adjusted HR for MI for individuals in the first vs fourth KIV-2 quartiles (Figure 3) yielded an instrumental variable estimate of the HR for MI for a doubling of genetically elevated lipoprotein(a) levels of 1.22 (95% CI, 1.09-1.37) in the CCHS (Figure 4).

COMMENT

We observed an increase in risk of MI with increasing levels of lipoprotein (a), as well as with decreasing numbers of lipoprotein(a) KIV-2 repeats associated with elevated levels of lipoprotein(a). The increase in risk of MI associated with genetically elevated levels of lipoprotein(a) was consistently seen in 3 large independent studies, including a prospective study of the general population, a cross-sectional study of the general population, and a case-control study. The KIV-2 genotype explained 21% and 27% of the total lipoprotein(a) concentration variation in the CCHS and the CGPS. Instrumental variable analysis (in which the increase in lipoprotein[a] levels explained by the KIV-2 genotype was related to MI) directly demonstrated that genetically elevated lipoprotein(a) is associated with increased risk of MI, like elevations in plasma lipoprotein(a). These findings are consistent with a causal association of elevated lipoprotein(a) levels with increased MI risk.

The instrumental variable estimate of the HR for MI for a doubling of genetically elevated lipoprotein(a) was nominally larger than the HR for MI for a doubling of plasma lipoprotein(a) levels. However, genotypes are invariant over time and can exert an effect on plasma lipoprotein(a) levels over a lifetime and thus potentially be superior (in risk prediction) to a single plasma measurement affected by passing endogenous or exogenous factors.[18] We showed that lipoprotein(a) levels correlated inversely with number of KIV-2 repeats in all age groups from 21 to 93 years, and within the same individu-

als at measurements separated by 10 years, indicating that the effect of KIV-2 genotype on lipoprotein(a) plasma levels is indeed lifelong. Another possible explanation for the difference in the HRs (instrumental variable estimate of the HR vs the HR observed for plasma lipoprotein[a] levels) might be that lipoprotein(a) particles with a small apolipoprotein(a) isoform are particularly harmful, as previously indicated.[4,19] This is supported by the observation that on adjustment for plasma lipoprotein(a) levels, the association of KIV-2 genotype with risk of MI in the CCHS was attenuated but remained significant.

Mechanistically it is plausible that elevated levels of lipoprotein(a) cause increased risk of MI. Lipoprotein(a) consists of a cholesterol-loaded LDL particle linked to a plasminogen-like glycoprotein, apolipoprotein(a), and this dual structure indicates that lipoprotein(a) may contribute to the development of not only atherosclerosis like LDL, but also thrombosis and thus MI.[9] Atherosclerotic plaques but not normal human arteries contain lipoprotein(a),[20-22] and results from in vitro and animal studies have implicated lipoprotein(a) in foam-cell formation, smooth muscle–cell proliferation, and plaque inflammation and instability.[20,21,23] Lipoprotein(a) can cross the endothelial barrier between plasma and the arterial intima[22,24] and may be trapped within the arterial intima, particularly at sites of injury.[25-27] Thus, lipoprotein(a) can deliver cholesterol to atherosclerotic plaques like LDL. Furthermore, lipoprotein(a) promotes thrombosis partly through competitive inhibition of plasmin generation and through inactivation of tissue factor pathway inhibitor, a potent inhibitor of the tissue factor–mediated coagulation cascade.[20,21]

We assessed the association of elevated plasma lipoprotein(a) levels with risk of MI solely in the CCHS. However, the association of plasma levels of lipoprotein(a) with risk of MI is supported by previous studies, including a meta-analysis of 18 studies reporting a risk ratio of 1.7 for ischemic heart disease when comparing individuals in the upper vs lower tertile of the lipoprotein(a) distribution.[2] In accordance with our findings, previous smaller case-control studies ($n < 2400$) have demonstrated an association of either apoalipoprotein(a) isoform size or *LPA* KIV-2 genotype with risk of cardiovascular disease, despite study sizes often being limited by the use of labor-intensive methods of KIV-2 phenotyping or genotyping (gel electrophoresis followed by Western or Southern blotting).[4,19,28-36] In the past, only these complicated methods were available for KIV-2 phenotyping or genotyping, and therefore large studies like ours have never been performed previously. Nevertheless, our findings are generally consistent with findings from the larger of the previous case-control studies that provided risk estimates for coronary heart disease for a phenotypically low number of KIV-2 repeats in the apolipoprotein(a) protein.[4,31-33,36] Only a few previous case-control studies have directly evaluated risk of disease according to KIV-2 genotype, and results were not consistent[34,37,38]; 2 case-control studies of white individuals demonstrated an association of a low number of KIV-2 repeats with risk of coronary heart disease,[34,37] while a case-control study conducted in an Asian Indian population did not.[38]

We benefitted from an in-house, high-throughput, real-time PCR assay developed for the present study to genotype a total of 40 486 individuals for the KIV-2 size polymorphism. The *LPA* gene codes for several kringle structures in addition to the KIV-2 repeats; kringle IV types 1 and 3 through 10 as well as a kringle V structure are encoded.[9] Because of a high degree of sequence homology between the different kringle structures and considerations pertaining to reported gene variation, the primer and probe design could not exclude the kringle IV type 1 repeat. However, the *LPA* gene has been extensively studied and only the KIV-2 repeat has ever been reported to occur in varying numbers.[9] Therefore, real-time PCR results were simply corrected for the KIV-1 contribution prior to statistical analysis. 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 lipoprotein(a) concentration, and furthermore, it includes nonexpressed alleles.[39] Despite these assay limitations, our KIV-2 genotype results explained 21% and 27% of the total interindividual variation in lipoprotein(a) concentration in 2 independent samples of the general population. However, these assay limitations may have led to more conservative MI risk estimates for decreased numbers of KIV-2 repeats.

The term *mendelian randomization* refers to the random assortment of genes from parents to offspring at the time of conception.[6-8,40] In genetic association studies of the general population, such as ours, the laws of mendelian genetics ensure that groups of individuals, defined by genotype, can be compared with a randomized controlled trial, in which the random assignment to a control or intervention group likely results in similar distributions of known as well as unknown confounders.[6,40] Thus, associations of genotype with risk of disease are generally unconfounded, with the exception of possible genetic confounding caused by population admixture or linkage disequilibrium.[6,40] It is unlikely that our results were confounded by population admixture since all participants in the present study were white individuals of Danish descent. Also, considering the stepwise increase in lipoprotein(a) levels and risk of MI with decreasing numbers of KIV-2 repeats, it seems unlikely that our results could be explained by linkage disequilibrium, that is, the association of the KIV-2 size polymorphism with other unknown genetic variation. Furthermore, a mendelian randomization study avoids associations based on reverse causation,[7] ie, the association of a bio-

chemical marker with risk of disease due to disease-induced increases in levels. This means that the association of elevated levels of lipoprotein(a) with risk of MI cannot be explained by atherosclerosis-induced increases in levels of lipoprotein(a). Also, in support of a causal association of elevated lipoprotein(a) levels with risk of MI, risk estimates for MI for elevated levels of lipoprotein(a) were attenuated on adjustment for the KIV-2 genotype.

A limitation of the present study is that we studied only white individuals drawn from the same relatively narrow population. Therefore, results may not apply to other ethnic groups, particularly because plasma levels of lipoprotein(a) differ between different ethnic groups.[9] Thus, replication of our findings in other populations would further strengthen the claim of causality for elevated lipoprotein(a) levels and increased MI risk. Also, information on end point was gathered since 1976, and changes in medications, procedures (ie, coronary artery bypass or stenting), and diagnostic criteria over time may have affected the registration of MI. This may partly explain the slight differences in risk estimates observed for the 3 studies.

The present study demonstrated an association between lipoprotein(a) level and MI, an association between KIV-2 genotype and lipoprotein(a) level, and an association between KIV-2 genotype and MI. Each of these associations is supported by evidence from previous studies. However, no previous studies have directly employed mendelian randomization approaches and instrumental variable analysis to assess consistency with causality. We now demonstrate, by the sheer size and statistical power of our study, an association between lipoprotein(a) KIV-2 genotype and MI risk in the general population consistent with a causal association of elevated lipoprotein(a) levels with increased MI risk. Nonetheless, final proof of causality still requires randomized clinical trials demonstrating reduced MI risk in response to lipoprotein(a)-lowering therapy.

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Author Contributions: Dr Kamstrup had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
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Acquisition of data: Kamstrup, Tybjærg-Hansen, Steffensen, Nordestgaard.
Analysis and interpretation of data: Kamstrup, Nordestgaard.
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Reason is the enumeration of quantities already known; imagination is the perception of the value of those quantities, both separately and as a whole. Reason respects the differences, and imagination the similitudes of things. Reason is to imagination as the instrument to the agent, as the body to the spirit, as the shadow to the substance.

—Percy Bysshe Shelley (1792-1822)