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Elevated Lipoprotein(a) and Risk of Aortic Valve Stenosis in the General Population

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Objectives The purpose of this study was to determine whether elevated lipoprotein(a) levels and corresponding *LPA* risk genotypes (rs10455872, rs3798220, kringle IV type 2 repeat polymorphism) prospectively associate with increased risk of aortic valve stenosis (AVS).

Background The etiologic basis of AVS is unclear. Recent data implicate an *LPA* genetic variant (rs10455872), associated with Lp(a) levels, in calcific AVS.

Methods We combined data from 2 prospective general population studies, the Copenhagen City Heart Study (1991 to 2011; n = 10,803) and the Copenhagen General Population Study (2003 to 2011; n = 66,877), following up 77,680 Danish participants for as long as 20 years, during which time 454 were diagnosed with AVS. We conducted observational and genetic instrumental variable analyses in a Mendelian randomization study design.

Results Elevated Lp(a) levels were associated with multivariable adjusted hazard ratios for AVS of 1.2 (95% confidence interval [CI]: 0.8 to 1.7) for 22nd to 66th percentile levels (5 to 19 mg/dl), 1.6 (95% CI: 1.1 to 2.4) for 67th to 89th percentile levels (20 to 64 mg/dl), 2.0 (95% CI: 1.2 to 3.4) for 90th to 95th percentile levels (65 to 90 mg/dl), and 2.9 (95% CI: 1.8 to 4.9) for levels greater than 95th percentile (>90 mg/dl), versus levels less than the 22nd percentile (<5 mg/dl; trend, $p < 0.001$). Lp(a) levels were elevated among carriers of rs10455872 and rs3798220 minor alleles, and of low number of KIV-2 repeats (trend, all $p < 0.001$). Combining all genotypes, instrumental variable analysis yielded a genetic relative risk for AVS of 1.6 (95% CI: 1.2 to 2.1) for a 10-fold Lp(a) increase, comparable to the observational hazard ratio of 1.4 (95% CI: 1.2 to 1.7) for a 10-fold increase in Lp(a) plasma levels.

Conclusions Elevated Lp(a) levels and corresponding genotypes were associated with increased risk of AVS in the general population, with levels >90 mg/dl predicting a threefold increased risk. (J Am Coll Cardiol 2014;63:470–7)

Lipoprotein(a) is considered a causal risk factor for ischemic cardiovascular disease (1–4). Recently, a genome-wide association study has identified a genetic variant (rs10455872 SNP) in the *LPA* gene locus, determining plasma levels of Lp(a), as associated with aortic valve calcium and aortic valve stenosis (AVS) (5).

Calcific AVS represents the most common heart valve condition requiring treatment among adults in Western societies (6). It is estimated to affect 2% to 7% of the population more than 65 years of age (7). Previously considered a passive, degenerative condition in which the valve degenerates with age in association with calcium accumulation, it is increasingly clear that disease progression is a regulated process that shares risk factors with atherosclerotic cardiovascular disease, including elevated cholesterol levels, hypertension, smoking, and diabetes mellitus (8–10). In addition, congenital malformations of the aortic valve, such as bicuspid valve morphology, possibly present in ~50% of patients with severe AVS, represent important risk factors (11). However, at present, and despite some shared risk factors with atherosclerotic disease, no means to effectively

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prevent aortic valve disease progression exists, and aortic valve replacement, costly and associated with perioperative mortality, remains the only treatment option (5,7). It is hoped that identification of causal risk factors may open opportunities for prevention.

Lp(a) consists of a cholesterol-laden low-density lipoprotein particle bound to a plasminogenlike glycoprotein, apolipoprotein(a) (12). Lp(a) promotes atherosclerotic stenosis, and possibly thrombosis, and has been hypothesized to contribute to wound healing (3,13–16), each of which could explain an association with AVS. Plasma levels of Lp(a) are primarily genetically determined by variation in the *LPA* gene coding for apolipoprotein(a) (12). Variation in the *LPA* gene includes the kringle IV type 2 (KIV-2) repeat polymorphism, determining the size of the expressed apolipoprotein(a), the size of which correlates inversely with Lp(a) levels (1,12). Two single nucleotide polymorphisms (SNPs), rs10455872 and rs3798220 tagging the KIV-2 polymorphism, also strongly associate with Lp(a) levels (2).

In the present study, we tested the hypothesis that elevated Lp(a) levels and corresponding *LPA* risk genotypes (low number of KIV-2 repeats, rs10455872 carriers, rs3798220 carriers) prospectively associate with increased risk of AVS in the general population. We assessed genetic evidence by conducting instrumental variable analysis in a Mendelian randomization study design (17). For these purposes, we combined data from 2 large prospective studies of the Danish general population, the CCHS (Copenhagen City Heart Study) and the CGPS (Copenhagen General Population Study), with baseline information on Lp(a) levels and *LPA* KIV-2 repeat, rs10455872, and rs3798220 genotypes.

Methods

Participants. The CCHS is a general population study initiated in 1976 to 1978 with follow-up examinations in 1981 to 1983, 1991 to 1994, and 2001 to 2003 (18). Participants were randomly selected from the Copenhagen Civil Registration System to reflect the general population of Copenhagen ages 20 to ≥100 years. Examinations included a self-administered questionnaire, reviewed by an investigator on the day of attendance, a physical examination, and blood sampling. Smokers were active smokers, and diabetes mellitus was defined as self-reported disease, use of insulin or oral hypoglycemic drugs, and/or a nonfasting plasma glucose of >11 mmol/l. For the present study, we included all CCHS participants of Danish descent with no prior history of AVS, and with an available Lp(a) measurement performed shortly after sampling: 9,647 from the 1991 to 1994 examination (16,563 invited, 61% response rate) and an additional 1,156 from the 2001 to 2003 examination (12,599 invited, 50% response rate). A blood sample for deoxyribonucleic acid analysis was available for a total of 10,359 included participants. Among included 1991 to 1994 participants, 4,629 also had Lp(a) measurements performed at the 2001 to 2003 examination, allowing correction for regression dilution bias (19).

Abbreviations and Acronyms

AVS = aortic valve stenosis
CI = confidence interval
ICD = International Classification of Diseases
KIV-2 = kringle IV type 2
SNP = single nucleotide polymorphism

The CGPS is a Danish general population study initiated in 2003 and still recruiting (45% response rate) (20). Participants of Danish descent were randomly selected from the Copenhagen Civil Registration System to represent the population of the greater Copenhagen area ages 20 to ≥100 years. Data collection in this study is almost identical to that of the CCHS. For the present study, we included all participants of Danish descent without prior history of AVS, and with an available *LPA* rs10455872, rs3798220, or KIV-2 genotype, and/or an available Lp(a) measurement (n = 66,877). Among included participants, *LPA* rs10455872 genotypes were available on 66,780 (recruited 2003 to 2010), rs3798220 genotypes on 66,803 (recruited 2003 to 2010), KIV-2 genotypes on 29,347 participants (recruited 2003 to 2006), and Lp(a) measurements on 18,213 participants (recruited 2003 to 2005).

We followed up all CCHS and CGPS participants from time of study inclusion and censored at the occurrence of AVS (n = 454), death (n = 6,632), emigration (n = 364), or May 2011, whichever came first. Follow-up was 100% complete; that is, we did not lose track of a single person during follow-up.

Information on diagnoses of AVS (International Classification of Diseases-Eighth Edition [ICD-8], codes 424.10, 424.12, 424.18, 424.19; and 10th edition [ICD-10], codes I35.0 and I35.2) were ascertained from the national Danish Patient Registry, and the national Danish Causes of Death Registry, public registers to which all hospitalizations and deaths in Denmark have been reported since 1977. Similarly, information on diagnoses of myocardial infarction (ICD-8 code 410; and ICD-10 codes I21 to I22) and congenital aortic valve malformations (ICD-8 codes 746.62, 746.89, 746.99, and 747.29; and ICD-10 codes Q23.0, Q23.1, Q23.8, and Q23.9) were ascertained from these registries for all participants. Among persons with a diagnosis of AVS, information on aortic valve replacement were ascertained from national classifications of surgical procedures (surgery and treatment procedures from 1971 to 1995, codes 30780, 30810, 31268, 31269; and SKS surgery procedures codes KFMD00 to KFMD20 and KFMD96) administered by the Danish National Board of Health.

The CCHS and the CGPS were approved by Herlev Hospital and by a Danish ethical committee, and were conducted according to the Declaration of Helsinki. Participants gave written informed consent.

Laboratory analysis. At the 1991 to 1994 CCHS examination, Lp(a) total mass was measured using a well-validated in-house assay, as described previously (21). At the 2001 to 2003 CCHS examination and for the first 5,576 CGPS

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measurements in 2003 to 2004, Lp(a) total mass was measured immediately after sampling using a sensitive immunoturbidimetric assay from DiaSys (DiaSys Diagnostic Systems, Holzheim, Germany), as described previously (21). An additional 12,637 Lp(a) total mass measurements were performed in 2011 on thawed samples from CGPS participants recruited in 2004 to 2005, and using a well-validated latex-enhanced immunoturbidimetric assay from Denka Seiken (Denka Seiken, Tokyo, Japan). Enzymatic assays were used on fresh samples to measure plasma levels of total cholesterol, high-density lipoprotein cholesterol, and apolipoprotein B. The *LPA* KIV-2 repeat polymorphism was genotyped by real-time polymerase chain reaction analysis yielding an estimate of the sum of repeats on both alleles, as described previously (1). Genotyping for *LPA* rs3798220 and rs10455872 SNPs was performed by TaqMan (Applied Biosystems, Foster City, California) analysis. Genotype distributions did not differ from those predicted by the Hardy-Weinberg equilibrium.

Statistical analysis. We used Stata SE 12.1 (StataCorp., College Station, Texas). One-way analysis of variance was used to estimate the contribution of *LPA* KIV-2, rs10455872, and rs10455872 genotypes to the variation in plasma Lp(a) levels (levels were square root transformed because of skewedness of the distribution).

For further analyses, participants were divided into groups based on percentiles of the Lp(a) concentration distribution, as done previously (where 5 mg/dl, corresponding to the 22nd percentile, represents the detection limit of our in-house assay) (1,21), or based on corresponding *LPA* KIV-2 percentiles, or on *LPA* rs10455872 and rs3758220 carrier status. To avoid potential bias from use of different Lp(a) assays (although comparable [21]), Lp(a) percentile groups were defined separately for each assay and then combined. Lp(a) cutpoints in mg/dl, corresponding to percentile cutpoints, are based on fresh sample Denka Seiken measurements (n = 22,000), a widely available commercial assay. Cuzick nonparametric test for trend was used to test for differences in Lp(a) levels between different *LPA* genotype combinations. Cox regression was used to estimate hazard ratios of incident AVS with 95% confidence intervals (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 total cholesterol, high-density lipoprotein cholesterol, systolic blood pressure, smoking, and diabetes mellitus. Information on additional covariates was ~99% complete; the few persons who lacked information on covariates were excluded from multivariable adjusted analyses. Total cholesterol values were adjusted for the Lp(a) contribution, as done previously (1). Based on the second Lp(a) measurement in 2001 to 2003, hazard ratios for increased Lp(a) levels were corrected for regression dilution bias (19). Proportionality of hazards over time was assessed by plotting the cumulative hazard (on a log scale) versus analysis time. Suspicion of nonparallel lines was further tested using Schoenfeld residuals. No major violations of the proportional hazards assumption were detected. Interaction of Lp(a) levels or *LPA* genotypes with other covariates including sex was evaluated by comparing models with and without 2-factor interaction tests using maximum likelihood ratio tests. No interactions were observed (e.g., p values for interaction with sex ranged from 0.15 [genotypes] to 1.00 [Lp(a) levels]). Finally, to validate findings for elevated plasma Lp(a) levels, sensitivity analyses with Cox regression were conducted.

Instrumental variable analysis, using 3 different approaches, was used to estimate relative risk or hazard ratio estimates of AVS for a 10-fold increase in genetically elevated Lp(a) levels (17). The *LPA* genotypes (rs10455872 genotype, rs3798220 genotype, and/or KIV-2 percentile groups) were used as instrumental variables, that is, variables that only affect outcome, namely, AVS, through a robust association with an intermediary variable, namely, Lp(a), and importantly, do not suffer from the same error terms as found in the direct association of the intermediary variable with risk of endpoint, including potential confounding and reverse causation. (For a detailed description of instrumental variable analyses, please refer to Methods in the Online Appendix.)

For comparison with instrumental variable analysis genetic risk estimates, we calculated the observational age- and sex-adjusted hazard ratio for AVS for a 10-fold increase in plasma Lp(a) levels.

Results

Baseline characteristics of the 77,680 participants (all and by lipoprotein[a] percentiles) in the combined CCHS and CGPS are shown in Table 1. (For baseline characteristics stratified by *LPA* genotypes, please refer to Online Tables 1 and 2.) We observed a total of 454 first-time AVS events during as much as 20 years (mean 5 years) of follow-up.

Lp(a) plasma levels and risk of aortic valve stenosis. Elevated Lp(a) levels associated with multivariable adjusted hazard ratios for AVS of 1.2 (95% confidence interval [CI]: 0.8 to 1.7) for 22nd to 66th percentile levels (5 to 19 mg/dl), 1.6 (95% CI: 1.1 to 2.4) for 67th to 89th percentile levels (20 to 64 mg/dl), 2.0 (95% CI: 1.2 to 3.4) for 90th to 95th percentile levels (65 to 90 mg/dl), and 2.9 (95% CI: 1.8 to 4.9) for levels more than 95th percentile (>90 mg/dl), versus levels less than 22nd percentile (<5 mg/dl; trend, $p < 0.001$) (Fig. 1). Corresponding events per 10,000 person-years were 11, 15, 18, and 24, respectively, versus 10 in the reference group.

In sensitivity analyses, we observed similar risk estimates in women and men separately (Online Fig. 1), when considering only AVS events resulting in aortic valve replacement (Online Fig. 2), when considering only AVS events recorded with recent ICD-10 coding (Online Fig. 3), when including only participants free of myocardial infarction (Online Fig. 4) or adjusting for such (Online Fig. 5), or when excluding participants diagnosed with congenital

Table 1 Baseline Characteristics of Participants at Study Entry

		Lp(a) Percentiles				
	All	<22nd (<5 mg/dl)	22nd–66th (5–19 mg/dl)	67th–89th (20–64 mg/dl)	90th–95th (65–90 mg/dl)	>95th (>90 mg/dl)
n	77,680	6,123	13,053	6,677	1,728	1,435
Women, %	56	51	55	55	58	62
Age, yrs	58 (47–67)	58 (47–68)	58 (47–68)	59 (48–69)	59 (48–69)	59 (50–69)
Total cholesterol, mg/dl	216 (189–247)	216 (185–247)	220 (193–251)	224 (197–255)	236 (205–263)	239 (212–274)
HDL cholesterol, mg/dl	62 (46–73)	62 (46–73)	58 (46–73)	62 (50–73)	62 (50–77)	62 (50–73)
Systolic BP, mm Hg	138 (125–152)	138 (124–152)	137 (124–152)	137 (125–152)	140 (125–155)	140 (125–155)
Smoking, %	24	35	34	33	34	34
Diabetes mellitus, %	4	5	4	4	5	5
Lp(a), mg/dl	14 (6–35)	3 (2–4)	11 (7–17)	40 (30–51)	80 (63–95)	124 (104–148)
LPA rs10455872, % carriers	14	1	1	31	59	45
LPA rs3798220, % carriers	2	2	1	1	6	24
LPA KIV-2, no. of repeats	36 (30–41)	38 (34–43)	37 (33–42)	33 (28–38)	28 (25–32)	27 (22–31)

Values are n, %, or median (interquartile range). Lp(a) levels were measured in 29,016 persons. To convert cholesterol (total and HDL) to mmol/l, multiply values by 0.0259.
BP = blood pressure; HDL = high density lipoprotein.

aortic valve malformations (Online Fig. 6). In analyses conducted separately for the CCHS and the CGPS, risk estimates were most pronounced in the CCHS with the longest follow-up (Online Fig. 7). For comparison with findings for Lp(a) levels, we evaluated risk of AVS as a function of apolipoprotein B quintiles or extreme levels and found no association (Online Figs. 8 and 9).

Genotypes and Lp(a) levels. In accordance with previous findings (2), median Lp(a) levels were 11 mg/dl, 60 mg/dl, and 108 mg/dl (trend, $p < 0.001$), respectively, for rs10455872 noncarriers, heterozygotes, and homozygotes, and correspondingly for rs3798220, 14 mg/dl, 95 mg/dl, and 133 mg/dl (trend, $p < 0.001$). Also, Lp(a) levels increased with decreasing numbers of *LPA* KIV-2 repeats: 8 mg/dl, 12 mg/dl, 23 mg/dl, 49 mg/dl, and 65 mg/dl (trend, $p < 0.001$), respectively, for number of KIV-2 repeats greater than the 79th percentile, between the 35th and 79th percentile, the 12th and 34th percentile, the sixth and 11th percentile, and less than the sixth percentile.

The rs10455872 genotype explained 28% of the total variation in plasma Lp(a) levels, the rs3798220 genotype explained 5%, and the KIV-2 genotype explained 24%. Combined, the genotypes explained 41% of the total variation in plasma Lp(a) levels. Carriers of rs10455872 and rs3798220 minor alleles had increased levels of Lp(a) across all categories of KIV-2 repeats (trend, $p = 0.04$ to $p < 0.001$), and Lp(a) levels increased with decreasing numbers of KIV-2 repeats across all rs10455872 and rs3798220 genotype categories (trend, all $p < 0.001$) (Fig. 2). Figure 2 demonstrates that although the genotypes are not

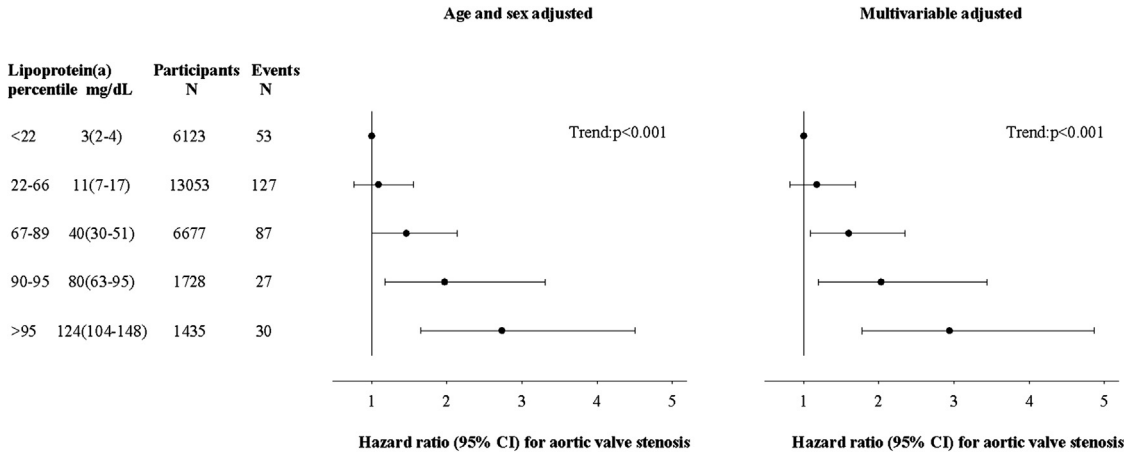

Figure 1 Risk of Aortic Valve Stenosis as Function of Elevated Lp(a) Levels

Analyses were adjusted for **(left)** age and sex or **(right)** multivariable adjusted additionally for total cholesterol, high-density lipoprotein cholesterol, systolic blood pressure, smoking, and diabetes mellitus. Lp(a) in mg/dl is shown as median (interquartile range). CI = confidence interval.

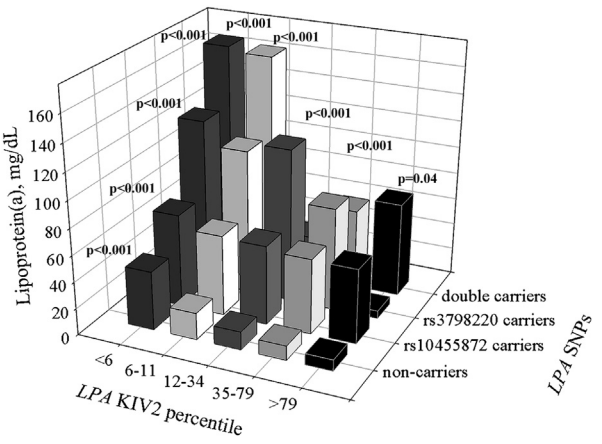

Figure 2 **Median Lp(a) Levels as Function of *LPA* Genotype**

The *LPA* KIV-2 genotype is displayed as percentile cutpoints of number of repeats. The *LPA* single nucleotide polymorphism (SNP) genotypes are displayed as noncarriers of both SNPs, carriers of only rs10455872 (including heterozygotes and homozygotes), carriers of only rs3798220 (including heterozygotes and homozygotes), and carriers of both SNPs (including heterozygotes and homozygotes for each SNP). The p values denote **(left)** test for trend of Lp(a) levels across KIV-2 percentile groups stratified on SNP genotypes, and **(right)** test for trend of Lp(a) levels across SNP genotypes stratified on KIV-2 percentile groups.

independent and that SNP carrier status is known to tag a relatively low number of KIV-2 repeats (2), all genotypes contribute independent information, for example, for a given number of KIV-2 repeats, SNPs associate independently with Lp(a) levels, and vice versa.

Genotypes and risk of aortic valve stenosis. Carriers of rs10455872 minor alleles were at increased risk of AVS with multivariable adjusted hazard ratios of 1.6 (95% CI: 1.2 to 2.0) and 1.5 (95% CI: 0.5 to 4.8) for heterozygotes and homozygotes as compared to noncarriers (trend, $p < 0.001$) (Fig. 3). Corresponding events per 10,000 person-years were 15 and 13, versus 10 in noncarriers. We found no increased risk of AVS in carriers of rs3798220 minor alleles with a hazard ratio of 1.0 (95% CI: 0.5 to 1.8) for heterozygotes as compared to noncarriers. Corresponding events per 10,000 person-years were 10 and 11. No homozygotes experienced an event. Finally, we found a trend toward increased risk of AVS for low number of KIV-2 repeats with, for example, hazard ratios of 1.1 (95% CI: 0.7 to 1.8) and 1.5 (95% CI: 0.9 to 2.3) for number of KIV-2 repeats between the sixth and 11th percentile, and less than sixth percentile, as compared to number of repeats more than 79th percentile (trend, $p = 0.06$). Corresponding events per 10,000 person-years were 14 and 17, versus 13 in the reference group.

Instrumental variable analysis, observational versus genetic estimates. A 10-fold increase in plasma Lp(a) levels associated with an age- and sex-adjusted hazard ratio of AVS of 1.4 (95% CI: 1.2 to 1.7), namely, the observational estimate (Fig. 4). In comparison, the instrumental variable analysis estimate of the relative risk of AVS for a 10-fold increase in genetically determined Lp(a) values was 1.6 (95% CI: 1.2 to 2.1) based on individual participant data and including all *LPA* genotypes (rs10455872 genotype, rs3798220 genotype, and KIV-2 percentile group; F-value of 1,238). We observed similar results using a combined genotype score or a combined extreme genotype score approach. In analyses based on each genotype separately, using individual participant data, only results for the rs10455872 genotype reached statistical significance.

Discussion

In the present prospective study of as many as 77,680 persons, we demonstrate dose-dependent increased risk of AVS in those with elevated Lp(a) levels, a novel finding in a general population setting. Persons with Lp(a) levels greater than the 90th percentile thus have a twofold to threefold increased risk of having AVS, as compared to persons in the lower fifth of the concentration distribution, and with similar findings in women and men separately. Further, the confirmed association of *LPA* rs10455872 minor allele carrier status with increased risk of AVS, taken together with our instrumental variable analysis results including information on 3 *LPA* genetic variants (all associated with lipoprotein[a] levels), indicate that the association may be causal.

Mechanism of action. Lp(a) is an emerging risk factor for ischemic cardiovascular disease (4,22), where data from in vitro, animal, and large genetic studies of *LPA* gene variants (1–3,13,14) provide evidence for a causal role in promoting atherosclerotic stenosis, and possibly also thrombosis at extreme Lp(a) levels. Lp(a) is found exclusively in humans, apes, and Old World monkeys, and in an aberrant form in the hedgehog (12). Thus, Lp(a) developed twice independently during evolution, yet the normophysiological role of Lp(a) is unknown. A role for Lp(a) in wound healing has been hypothesized (12,15), where Lp(a) may bind to fibrin through its apolipoprotein(a) component, possibly inhibiting fibrinolysis limiting bleeding, and may deliver cholesterol through its low-density lipoprotein component to sites of tissue healing. This hypothesis is supported by data demonstrating preferential accumulation of Lp(a) at sites of tissue injury and repair (16,23). Both Lp(a)-mediated mechanisms involved in normophysiological phenomena such as wound healing and in pathophysiological manifestations such as atherosclerotic stenosis may explain an association with AVS, considered an ongoing process of injury and tissue repair responses, and sharing risk factors with atherosclerotic disease (6,8,9). Incidentally, apolipoprotein (a), B, and E have been demonstrated in early through end-stage aortic valve lesions (but not in unaffected valves) in humans (24).

An alternative explanation for the association of Lp(a) levels with risk of AVS, may be AVS with or without underlying congenital malformations and resulting

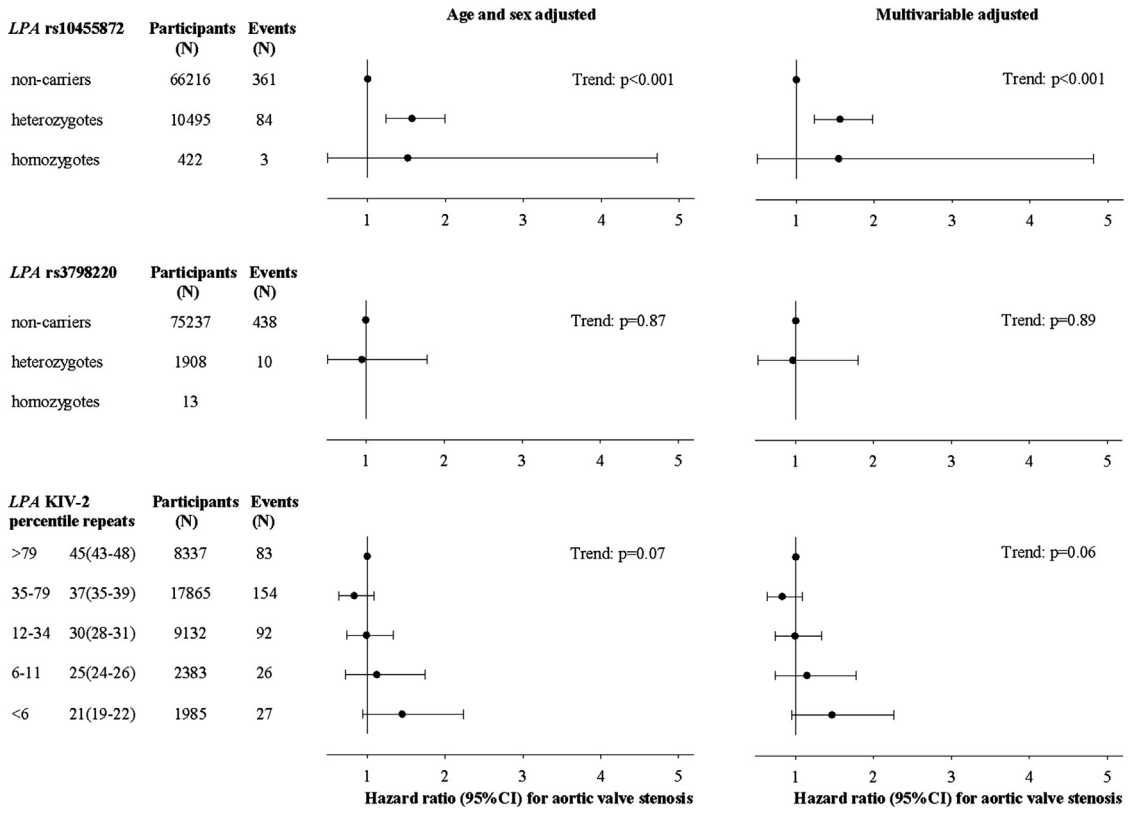

Figure 3 Risk of Aortic Valve Stenosis as Function of *LPA* rs10455872, rs3798220, and KIV-2 Genotypes

The *LPA* KIV-2 genotype is displayed as percentile cutpoints of number of repeats, also shown as median (interquartile range) for number of repeats. Analyses were adjusted for **(left)** age and sex or **(right)** multivariable adjusted additionally for total cholesterol, high-density lipoprotein cholesterol, systolic blood pressure, smoking, and diabetes mellitus. CI = confidence interval.

post-stenotic turbulent flow leading to Lp(a) elevations, possibly also dependent on isoform size. However, AVS is not capable of altering a person's *LPA* genotype, and thus, reverse causality can not explain the association of genotype with AVS. Further, for the association of *LPA* genotypes with risk of AVS to be confounded by, for example, a concomitant bicuspid valve, the *LPA* genotypes must be in linkage disequilibrium with genes implicated in congenital bicuspid valve development, which to the best of our knowledge has never been reported. Also, given the stepwise association of *LPA* rs10455872 SNP and number of KIV-2 repeats with risk of AVS, confounding of the association by linkage disequilibrium seems highly unlikely.

Comparison with previous studies. In support of our general population findings, an association of elevated Lp(a) levels with increased risk of AVS or aortic valve sclerosis, considered a precursor of AVS (7) has been reported in smaller case-control studies (25–27) and in an elderly population, where those with levels greater than the 75th percentile were at 23% increased risk of primarily aortic valve sclerosis as compared to those with levels less than the 25th percentile in cross-sectional analyses (10). Recently, a genome-wide association study has identified the *LPA* rs10455872 minor allele as being associated with presence of aortic valve calcium, a possible early phenotype for valvular heart disease (5). Instrumental variable analysis using this variant only, and conducted in a subset of participants, suggested causality between elevated Lp(a) levels and aortic valve calcium. We contributed confirmatory data (i.e., rs10455872 genotypes in the CCHS) to this study, demonstrating an association of rs10455872 with clinical AVS in the CCHS (5). We now extend previous findings, and in our combined study of the CCHS and the CGPS, demonstrate a clear stepwise increase in risk of AVS with increasing levels of Lp(a) in the general population. Further, we provide genetic data using information on 3 *LPA* gene variants explaining 41% of the total variation in plasma Lp(a) levels, and demonstrating a 1.6-fold increased risk of AVS upon a 10-fold increase in genetically elevated Lp(a) levels; importantly, Lp(a) levels may vary 1,000-fold between individual persons (12). Of note, the rarity of rs3798220 carrier status severely limited our ability to predict any increased risk of AVS on the basis of this genotype alone.

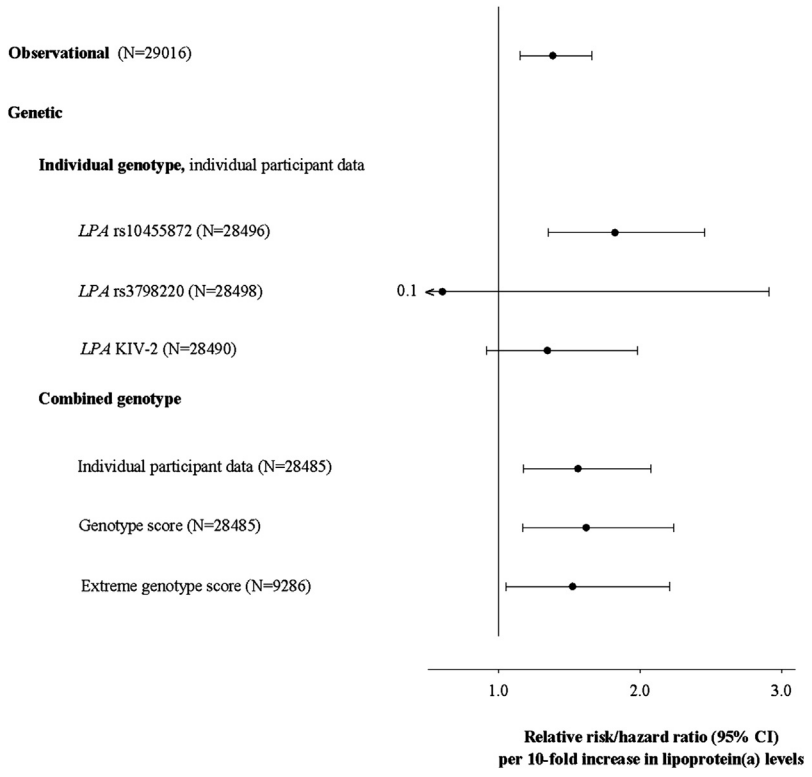

Figure 4 Instrumental Variable Analysis

Observational and genetic risk estimates for aortic valve stenosis for a 10-fold increase in Lp(a) levels. CI = confidence interval.

In contrast to findings for aortic valve disease, elevated Lp(a) levels do not seem to play a major role in coronary artery calcification associated with low-density lipoprotein cholesterol and apolipoprotein B levels, as demonstrated in a recent study (28), and this may reflect differences in pathobiology of valvular vs. vascular calcification. Also, we found no association of apolipoprotein B levels with risk of AVS.

Study limitations. We included solely white persons of Danish descent, which may limit the generalizability of our findings, especially as Lp(a) levels vary with ethnicity (12). However, the uniformity of the included participants ensures minimal risk of confounding by population admixture, which if present, may lead to false positive findings in instrumental variable analyses (17). Ascertainment of AVS was done by ICD-8 and ICD-10 codes and not by prospective clinical outcome data collection or by valve imaging of all participants. A limitation of such an approach is that, in particular, ICD-8 codes may not be entirely specific for AVS, that AVS events may include both calcification of a trileaflet valve and a congenitally bicuspid valve as these 2 conditions may be indistinguishable with severe disease, and further, that participants with less severe aortic stenosis may not have been coded as having AVS. However, such misclassification of participants with or without AVS may only have resulted in an underestimation of the true strength of the association. In further support of the validity of our findings, sensitivity analyses revealed similar results when only including events leading to aortic valve replacement, when only including events recorded with recent ICD-10 coding, when excluding participants diagnosed with myocardial infarction where high Lp(a) levels represent a contributing factor (1,2), or when excluding participants diagnosed with congenital aortic valve malformations, although likely underdiagnosed in our general population sample. Of note, overall participants with congenital aortic valve malformations did not have elevated Lp(a) levels (data not shown).

Conclusions

We demonstrate stepwise increases in risk of AVS with increasing Lp(a) levels in the general population. Further, we demonstrate association of genetic variation affecting plasma levels of Lp(a) with risk of AVS. However, to firmly establish whether elevated Lp(a) causes increased risk of AVS, evidence from clinical trials of the effect of lowering Lp(a) levels is required.

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APPENDIX

For supplemental methods, references, figures, and tables, please see the online version of this article.