Genetic variation in *LPA* and calcific aortic valve stenosis in patients undergoing cardiac surgery and familial risk of aortic valve microcalcification

Brief title: LPA variants and calcific aortic valve stenosis

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Key points

Question: Does genetically-elevated Lipoprotein(a) (Lp[a]) predict calcific aortic valve stenosis (CAVS) in patients independently of coronary artery disease (CAD) and do first-degree relatives of patients with CAVS and high Lp(a) levels are more likely to be characterized by aortic valve microcalcification?

Findings: Genetically-elevated Lp(a) was strongly association with CAVS, regardless of the presence/absence of CAD and first-degree relatives of patients with CAVS and high Lp(a) levels were more likely to have CAVS and/or aortic valve microcalcification.

Meaning: Lp(a) is an independent risk factor for CAVS and measuring Lp(a) could be useful to identify individuals at risk of future CAVS.

Abstract

Importance: Genetic variants at the *LPA* locus are associated with both calcific aortic valve stenosis (CAVS) and coronary artery disease (CAD). Whether these variants predict CAVS in patients with versus without CAD is unknown.

Objectives: 1) To test the association between *LPA* variants and CAVS in a cohort of patients undergoing heart surgery, 2) to study the association between *LPA* and CAVS in patients with vs. without CAD and 3) to determine whether first-degree relatives of patients with CAVS and high lipoprotein(a) (Lp[a]) levels showed evidence of aortic valve microcalcification.

Design: Genetic association study and family study.

Setting: The genetic association study includes patients undergoing cardiac surgery or participants from the general population and the family study includes first-degree relatives of patients with CAVS.

Participants: 1009 CAVS cases and 1017 controls undergoing cardiac surgery, 3258 cases and 41,100 controls with CAD, 2069 cases and 380,075 controls without CAD and 33 first-degree relatives of 17 patients with both CAVS and high Lp(a) (≥125 nmol/L) and 23 controls with Lp(a) levels (<125 nmol/L).

Exposures: Case-control studies.

Main Outcomes and Measures: Presence of CAVS according to a weighted genetic risk score (wGRS) based on three common Lp(a)-raising variants and presence of aortic valve

microcalcification, defined as the mean tissue-to-background ratio (TBR) ≥1.25 measured by 18F-NaF positron emission tomography/computed tomography.

Results: In QUEBEC-CAVS, each SD increase of the GRS was associated with a higher risk of CAVS [OR=1.35 (95% CI: 1.10-1.66), P=0.003]. Each SD increase of the GRS was associated with a higher risk of CAVS in patients with CAD [OR=1.30 (95% CI: 1.20-1.42), P<0.001] and without CAD [OR=1.33 (95% CI: 1.14-1.55), P<0.001]. The percentage of individuals with a TBR≥1.25 or CAVS was higher in first-degree relatives of patients with CAVS (48.5%) and high Lp(a) compared to controls (13%, p=0.006).

Conclusions and Relevance: Genetically elevated Lp(a) level is associated with CAVS, independently of the presence/absence of CAD. First-degree relatives of patients with CAVS and high Lp(a) show evidence of aortic valve microcalcification, a finding that supports further research on the potential usefulness of cascade screening in this population.

Introduction

Lipoprotein(a) [Lp(a)] is an atherogenic lipoprotein particle that consists of a cholesterol rich particle analogous to low-density lipoprotein (LDL), where apolipoprotein B-100 is linked to apo(a) by a disulfide bond (1). In contrast to LDL, the Lp(a) particle carries a substantial amount of pro-inflammatory and pro-calcifying oxidized phospholipids (OxPL), which bind to both the apo(a) and the LDL moieties of Lp(a) (2). The circulating level of Lp(a) is largely determined by genetic variation at the *LPA* locus, which is highly polymorphic. One of the strongest determinants of Lp(a) levels is a copy number variation (CNV) at this locus encoding Kringle IV – type 2 (KIV-2) repeats, which influence the length of apo(a) (smaller isoform size being linked to higher plasma Lp(a) levels) (3). It has been estimated that up to 70-90% of the variance in circulating Lp(a) may be explained by genetic variations at the *LPA* locus (4).

Approximately 10 years ago, a series of genetic association studies revealed an association between variants associated with high Lp(a) levels and the risk of coronary artery disease (CAD) (5-7). Subsequent characterization of the *LPA* locus and it association with CAD risk makes it one of the strongest, most consistent and best-characterized locus associated with CAD risk (8,9). Investigating the genetics of aortic valve calcium (AVC), Thanassoulis et al. (10) identified *LPA* as the strongest genetic factor associated with the presence of AVC in an analysis of the Cohorts for Heart and Aging Research in Genetic Epidemiology (CHARGE) consortium. Population-based studies and hospital cohorts later revealed a strong link between Lp(a) and calcific aortic valve stenosis (CAVS) risk and progression (11-14).

CAVS is the most common form of valvular heart disease and its prevalence is steadily increasing in Western societies, affecting approximately 2% of the population older than 65 (15). Similar to atherosclerotic cardiovascular diseases, the molecular mechanisms that initiate CAVS

include infiltration of oxidized lipids and lipoproteins (such as oxLDL and Lp[a]) and inflammatory cells (such as macrophages and T cells) as well as extra-cellular matrix remodeling and calcification. CAD and CAVS also share similar clinical risk factors such as male sex, age, smoking, type 2 diabetes, hypercholesterolemia and elevated Lp(a). Results of our recent analysis also suggested that risk factors associated with ideal cardiovascular health (an unhealthy diet, physical inactivity, smoking, obesity, type 2 diabetes, hypertension and hypercholesterolemia), often referred to as Life's Simple 7, were associated with CAVS incidence in a large population-based study (16). While there is considerable overlap between pathobiological mechanisms and clinical risk factors between CAD and CAVS, whether this overlap also exists for genetic factors is currently unknown. Also unknown is whether the association between genetically-elevated Lp(a) levels and CAVS is independent of the presence of CAD is unknown.

Although, the association between Lp(a) and CAVS is strong and consistent, there is currently little evidence supporting the routine assessment of Lp(a) levels in patients with CAVS. Routine measurement of Lp(a) levels in patients with CAVS could assist in the identification of family members of patients with CAVS and high Lp(a) levels who could be at increased risk of developing CAVS in the future. Whether first-degree relatives of patients with CAVS and high Lp(a) levels may show early signs of aortic valve disease or are at increased CAVS risk is unknown.

The objective of this study was to 1) determine the association between genetically elevated Lp(a) levels and CAVS in a cohort of patients undergoing a heart surgery (including controls with CAD), 2) determine whether the association between genetically elevated Lp(a) levels and CAVS was observed in patients with and without CAD and 3) to determine whether first-degree relatives of patients with CAVS and high Lp(a) levels were characterized by aortic valve macro

or microcalcification assessed by a newly developed aortic valve imaging technique that assess active aortic valve microcalcification: 18F-NaF positron emission tomography (PET)/computed tomography (CT).

Methods

Study populations

This analysis includes 1009 cases and 1017 controls from the QUEBEC-CAVS study, 1350 cases and 349,043 controls from the UK Biobank, 508 cases and 20,421 controls from the EPIC-Norfolk study, 3469 cases and 51,711 controls from the GERA study as well as 3123 cases and 6530 control from three French cohorts. The characteristics of these cohorts are described in detail in the Supplementary data file. In the QUEBEC-CAVS cohort, patients with a history of myocardial infarction, coronary artery stenosis on coronary angiography, or documented myocardial ischemia were included in the CAD group. In the UK Biobank, patients with self-reported myocardial infarction (n=950), CAD from ICD or OPCS codes (n=25,162) were included in the CAD group. In the EPIC-Norfolk study, patients with documented myocardial ischemia or myocardial infarction or other ischemic heart disease on hospital admission were included in the CAD group. In GERA, CAD cases were defined using a diagnosis of myocardial infarction or CAD (ICD9 410-414); procedure codes for percutaneous coronary intervention or coronary artery bypass surgery.

The design and characteristics of the family study is also described in the Supplementary data file. Briefly, a series of consecutive patients with mild to severe CAVS who did not undergo AVR were recruited at the echocardiography lab of the IUCPQ. Lp(a) levels were measured in patients with CAVS and first-degree relatives of patients with CAVS and Lp(a) levels ≥125

nmol/L (brothers, sisters or children aged above 40) as well as a control group of participants with Lp(a) levels <125 nmol/L unrelated to patients with CAVS underwent aortic valve microcalcification assessment, as described in the Supplementary data file.

Genotyping, single-nucleotide polymorphisms selection and Lipoprotein(a) measurements

The associations between the selected single nucleotide polymorphism (SNPs) and Lp(a) levels were obtained from the study of Emdin *et al.* (8) in which SNPs effect on Lp(a) levels were estimated in European ancestry in the ARIC cohort (n= 2,758). SNPs that were selected for this study are presented in Supplementary Table 1. Beta coefficients are expressed in 1 SD (28 mg/dl) increase of Lp(a) level. We selected SNPs with a minor allele frequency superior to 1%. Only two SNPs (rs10455872, rs3798220) were available from the EPIC-Norfolk study. A weighted genetic risk score (wGRS) was calculated in each cohort by adding the number of Lp(a) raising alleles that the person had inherited at each variant that was included in the score, weighted by the effect of each variant on Lp(a) levels reported by Emdin *et al. (8). In the family study,* plasma Lp(a) levels were measured by a turbidimetric assay using the Tina-quant Lipoprotein(a) Gen.2 system (Cobas integra 400/800, Roche Diagnostics, Mannheim, Germany).

Statistical analyses

Participants characteristics were summarized by disease status for each study, and differences were assessed using two sample t-test or Pearson's chi-square test for continuous and discrete characteristics, respectively. Welch's t-test were used for samples who have unequal variances tested with F-test. All continuous variables were tested for normal distribution with the Shapiro-Wilk test. In cases the null hypothesis that the data are normally distributed was rejected, the data were log transformed for statistical analyses and presented as the median and interquartile range. The association between the wGRS and CAVS was tested using logistic regression analyses that were adjusted for age, sex and principal components, when available. In the

GERA cohort, genetic variants were modelled using PLINK2 in logistic regression models adjusted for age, age squared, sex and the 10 principle components. We performed a random-effect meta-analysis using the inverse-variance weighted method as implemented in rmeta package version 3.0 in R version 3.5.1. Sensitivity analysis were performed in the QUEBEC-CAVS cohort using only participants with CAVS and CAD compared to controls with CAD, and in the other cohorts using participants with CAVS with/without CAD compared to controls with/without CAD (except for the French cohort which did not had CAD status in controls). The frequency of participant with CAVS and TBR ≥1.25 in first-degree relative compared to controls with low Lp(a) levels were assessed using Pearson's chi-square test and the differences in mean TBR between first-degree relatives and participants in the control group were tested using two sample T-tests.

Results

Genetic association study

The association between the *LPA* wGRS and CAVS is presented in Figure 1. In QUEBEC-CAVS, each SD increase of the GRS was associated with a higher risk of CAVS [OR=1.35 (95% CI: 1.10-1.66), P=0.003]. The wGRS was also positively associated with CAVS in sensitivity analyses performed in patients with (3258 cases and 41,100 controls) and without CAD (2069 cases and 380,075) in cohorts who included individuals with and without CAD. Each SD increase of the wGRS was associated with a higher risk of CAVS in patients with CAD [OR=1.30 (95% CI: 1.20-1.42), P<0.001] and without CAD [OR=1.33 (95% CI: 1.14-1.55), P<0.001]. In the QUEBEC-CAVS cohort, each SD increase of the wGRS was associated with a higher risk of CAVS in patients with CAD only [OR=1.68 (95% CI: 1.33-2.12), P<0.001]. That analysis included 586 cases and 989 controls, all with CAD.

Because CAD status was not available for controls included in the French cohort, results of the French populations are not presented in this sensitivity analysis (Figure 1). The association between the *LPA* wGRS and CAVS in cases of the French population stratified on the presence/absence of CAD is presented in Figure 2. This analysis shows that the wGRS is associated with CAVS in patients with both CAVS and CAD [OR=1.69 (95% CI: 1.35-2.12), P<0.001] and in patients with CAVS only [OR=1.60 (95% CI: 1.18-2.16), P<0.001] compared to population controls.

Family study

The clinical characteristics of the 17 patients with CAVS, 33 of their first-degree relatives (including 6 with CAVS and 27 without CAVS) and a control group of 23 individuals with low Lp(a) levels is presented in Table 1. Clinical characteristics of first-degree relatives and controls with low Lp(a) levels were comparable, except for the percentage of CAD, lipoprotein(a) levels, transvalvular pressure gradients and aortic valve area.

The percentage of participants with either CAVS or a TBR≥1.25 among first-degree relatives of patients with CAVS and high Lp(a) levels and the control groups is presented in Figure 3A. The percentage of individuals with a TBR≥1.25 or CAVS was higher in first-degree relatives of patients with CAVS (48.5%) and high Lp(a) compared to controls (13%, p=0.006). No differences between the two groups were observed when we evaluated AVC for 18F-NF PET/CT by CT in first-degree relatives (data not shown). After excluding first-degree relatives with CAVS, the mean TBR of first-degree relatives (without CAVS) was higher compared to controls with low Lp(a) levels (Figure 3B).

Discussion

In this study, we found that genetic variation at the *LPA* locus associated with higher Lp(a) levels was strongly associated with CAVS risk in patients undergoing cardiac surgery. Investigating the association between a wGRS associated with high Lp(a) levels in four CAVS cohorts stratified for the presence of CAD, we found that the association between genetically-elevated Lp(a) levels and CAVS was independent of CAD status. Results of this study support the notion that the association between Lp(a) and CAVS is not mediated by the concomitant presence of CAD and that individuals with high Lp(a) levels might have high CAVS risk, even if they do not have CAD. In a proof-of-concept study, we found that first-degree relatives of patients with CAVS and elevated Lp(a) levels may be at higher risk of aortic valve microcalcification measured by 18F-NaF PET/CT compared to controls with lower Lp(a) levels. These results consolidate the strong and independent association of Lp(a) with CAVS and support further research about the potential usefulness of routine measurement of Lp(a) levels in patients with CAVS and cascade screening for Lp(a) in the setting of CAVS (Figure 4).

It is well recognized that the presence of CAD might influence symptomatology of CAVS and therefore the diagnosis of the disease, which is an important aspect to consider in genetic association studies of CAVS. Indeed, patients with CAD may become symptomatic at the stage of moderate aortic stenosis, whereas patients with severe aortic stenosis may remain asymptomatic in the absence of CAD. Another potential confounding factor is concomitant AVR of a severe or moderate CAVS in patients undergoing coronary artery bypass surgery. In the setting of moderate aortic stenosis, AVR is thus generally driven by the diagnosis of CAD and indication of coronary artery bypass. It must be recognized however that the presence of CAD relied on data from electronic medical records and was not validated by an independent committee in most of the cohorts. Additionally, study participants with subclinical CAD or

symptomatic CAD without hospitalization might not have been captured in several cohorts and could have been assigned to the non-CAD group. Combined with previous work supporting a potentially causal role for Lp(a) in the etiology of CAD (5,17), our finding that genetically elevated Lp(a) levels predict CAVS irrespective of the presence/absence of CAD support the notion that Lp(a) might be a strong risk factor and an important trigger for both coronary artery- and aortic valve diseases. Altogether, these observations provide additional support to the hypothesis that Lp(a) may represent a therapeutic target of interest for CAVS.

CAVS is a complex disease that was once thought to be the result of naturally occurring degenerative processes associated with aging. However, results of molecular and genetic association studies suggest that calcification of the aortic valve is a very tightly regulated process and that both genetic and environmental factors are pivotal for these processes (18). Imaging studies have also shown that years before the appearance of aortic calcification and anatomical disturbances of the aortic valve, the aortic valve may undergo a series of pro-inflammatory and osteogenic processes associated with early CAVS (19). The most compelling evidence that highlighted a genetic component in CAVS came from two studies performed in France investigating the regional distribution of patients undergoing aortic valve replacement and the familial aggregation of the disease concluded that CAVS has a strong genetic basis (20,21). More recently, Martinsson et al. (22) reported the results of a very large investigation of more than 6 million Swedish siblings and showed that the odds of developing CAVS were 3-fold higher if one had a sibling with CAVS and more than 30-fold higher if one had two siblings with CAVS. The association between Lp(a) levels and family risk of CAVS was however not reported in these studies. Another recent investigation by Verweij et al. (23) included patients with atherosclerotic cardiovascular diseases (ASCVD) and their first-degree relatives and found that first-degree relatives of patients with ASCVD and Lp(a) levels ≥50 mg/dL (approximately 125 nmol/L) were twice as likely to be characterized by coronary artery calcium accumulation or to

have had a cardiovascular event compared to those with lower Lp(a) levels. The link between Lp(a) levels and AVC accumulation was not reported in this family study.

The National Heart, Lung and Blood Institute Working Group to Reduce Lp(a)-Mediated Risk of CAD and CAVS recently included in its top priorities for Lp(a) research that clinical CAVS studies be performed to evaluate the role of Lp(a) in calcification with established techniques or with emerging techniques such as 18F-NaF PET/CT (24). Although previous studies have linked Lp(a) with aortic valve macrocalcification measured by CT (10,25), in this study, we used 18F-NaF PET/CT to assess microcalcification in first-degree relatives of patients with CAVS and high Lp(a) levels. The degree of CT-measured valve calcification reflects the amount of calcium regardless of when and at which rate the mineralization occurred in the course of CAVS, whereas the 18F-NaF PET/CT estimates the active mineral deposition in the aortic valve at a given time point. Therefore, in contrast to CT, which detects quiescent macrocalcification, 18F-NaF PET/CT binds to active calcified nodules through chemical reactions with hydroxyapatite, a crystalline structure of calcium and phosphates (26). This technique is accurate and reproducible to detect and quantify inflammation (18F-fluorodeoxyglucose uptake) and mineralization activity (18F-NaF uptake into aortic valve hydroxyapatite) (27-30). Both mineralization and inflammation are increased in patients with versus without CAVS, and the activity of both rises steadily with increasing disease severity, as recently described by Dweck et al (27). However, mineralization is the culprit pathological process of CAVS and the measurement of valvular 18F-NaF uptake by PET/CT might provide a novel biomarker of early disease activity and progression. Although active microcalcification assessed by 18F-NaF PET/CT is a predictor of future AVC, it must be emphasized that although our findings suggest that first-degree relatives of patients with CAVS and high Lp(a) levels have higher odds of being characterized by active microcalcification compared to controls with low Lp(a) levels, it is not possible to determine how many of them will show accumulation of AVC or develop clinically significant CAVS in the future. Long-term follow-

up of these individuals will help determine whether active microcalcification, in the setting of high Lp(a) levels will translate into macroscopic AVC accumulation measured by CT. Additionally, because of our limited samples size, we could not determine whether these individuals have higher levels of aortic valve microcalcification because they have high Lp(a) levels on average or because they have family history of CAVS.

In an effort to identify patients with high Lp(a) levels who may be at risk, the European Atherosclerosis Society Consensus Panel recommended measuring Lp(a) levels in individuals with premature CAD (or with a family history of premature CAD), familial hypercholesterolemia, recurrent CAD despite statin treatment or intermediate cardiovascular risk (31). Although it is tempting to suggest that Lp(a) levels should be assessed routinely in patients with CAVS, in the absence of guidelines about specific actions to be taken once a patient with CAVS and high Lp(a) is identified, this recommendation would be premature. CAVS is the most common form of heart valve diseases. Surgical replacement of the aortic valve is the only effective treatment for CAVS. Given the strong association between Lp(a) and CAVS risk (independent of the presence/absence of CAD), determining whether lowering Lp(a) levels in patients with mild-to-moderate CAVS would reduce the progression of CAVS or risk of future AVR for severe CAVS would be the ultimate proof of causal association between Lp(a) and CAVS while simultaneously opening new therapeutic options to treat patients with CAVS and high Lp(a) levels. Results of our study reinforce the notion that cascade screening for Lp(a) (the measurement Lp(a) levels in patients with CAVS as well as in their first-degree relatives) could be clinically useful. In light of failed statin trials in CAVS, whether targeting Lp(a) levels in these individuals would reduce future AVC accumulation or long-term CAVS risk should be tested in further studies. To our knowledge, only one agent (an antisense oligonucleotide targeting *LPA*) specifically designed to lower Lp(a) levels has been tested in a RCT. Viney et al. (32) reported that treatment with

AKCEA-APO(A)L_{RX} was associated with a dose-response reduction in plasma Lp(a) levels of up to 80-90% in healthy individuals with Lp(a) levels equal or above 60 mg/dL.

In conclusion, this investigation provides new genetic findings supporting an important role of Lp(a) in the aetiology of CAVS that is independent of the presence/absence of CAD. Although the impact of Lp(a)-lowering in patients with mild-to-moderate CAVS or patients at high CAVS risk still has to be established, our results provide evidence that the routine assessment of Lp(a) levels in patients with CAVS as well as in their first-degree relatives could be clinically useful.

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Table 1. Clinical characteristics of patients with CAVS, their first-degree relatives including those with and without CAVS and a control group with low Lp(a) levels included in the family study.

	Patients with CAVS	**All first-degree relatives**	**First-degree relatives without CAVS**	**Controls with low Lp(a) levels**
n	17	33	27	23
Age (year)	71.6 (7.7)	63.9 (8.8)	63.1 (8.7)	60.6 (5.8)
Male	64.7 (11)	66.7 (22)	63.0 (17)	60.9 (14)
Body mass index (kg/m^2)	30.8 (5.4)	29.3 (4.8)	29.4 (5.1)	28.1 (4.5)
Hypertension	94.1 (16)	54.6 (18)	51.9 (14)	34.8 (8)
Diabetes mellitus	23.5 (4)	18.2 (6)	14.8 (4)	8.7 (2)
Coronary artery disease	64.7 (11)	24.2 (8)*	22.2 (6)	4.4 (1)
Stroke	0 (0)	0 (0)	0 (0)	4.4 (1)
Active smokers	0 (0)	9.1 (3)	11.1 (3)	13.0 (3)
Lipid-lowering therapy	70.6 (12)	54.6 (18)	51.9 (14)	34.8 (8)
Aortic valve peak gradient (mmHg)	38.9 (17.3)	17.3 (16.9)*	10.3 (4.8)#	7.7 (2.4)
Aortic valve mean gradient (mmHg)	21.8 (10.5)	9.4 (9.6)*	5.4 (2.6)#	4.2 (1.3)
Aortic valve area (cm^2)	1.07 (0.39)	1.96 (0.74)*	2.20 (0.60)	2.41 (0.49)
Lipoprotein(a) (nmol/L)	194.6 (161.0-228.8)	76.4 (5.7-165.0)*	77.0 (7.5-180.7)#	6.5 (4.2-10.0)

Data are presented as mean (SD), % (n) or median (IQR)

* $p<0.05$ for First-degree relatives vs controls with low Lp(a)

# $p<0.05$ for First-degree relatives without CAVS vs controls with low Lp(a).

Figures Legend

Figure 1. Association of genetically-elevated Lp(a) levels with calcific aortic valve stenosis in patients with and without coronary artery disease.

Figure 2. Association of genetically-elevated Lp(a) levels with calcific aortic valve stenosis in patients with and without coronary artery disease in participants of the French cohorts compared to controls (with unknown CAD status).

Figure 3. Distribution of participants with calcific aortic valve stenosis or tissue-to-background ratio ≥1.25 in the family study (A) and mean aortic valve tissue-to-background ratio in first degree relatives without CAVS and controls with low Lp(a) levels (B).

Figure 4. Results of this study that included cohorts from four countries suggest that the association between genetically elevated Lipoprotein(a) levels and calcific aortic valve stenosis is comparable in patients with versus without coronary artery disease and that first-degree relatives of patients with high Lipoprotein(a) levels and calcific aortic valve stenosis may have higher aortic valve calcification and could be at higher future risk of aortic valve calcification or calcific aortic valve stenosis.