ORIGINAL RESEARCH

Lipoprotein(a) and the Apolipoprotein B/A1 Ratio Independently Associate With Surgery for Aortic Stenosis Only in Patients With Concomitant Coronary Artery Disease

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***Background*—**Aortic stenosis (AS) has different clinical phenotypes, including AS with or without concomitant coronary artery disease (CAD). It is unknown whether these phenotypes share the same risk factors. In particular, lipoprotein(a) [Lp(a)] and apolipoproteins (Apo) are associated with AS, but it is unknown whether these associations differ among phenotypes. In this prospective analysis we examined the impact of Lp(a) and Apo in subgroups of patients with AS.

***Methods and Results*—**We identified 336 patients (mean age at survey 56.7 years, 48% female) who underwent surgery for AS after a median 10.9 years (interquartile range 9.3 years), participants in 1 of 3 large population surveys. For each patient, 2 matched referents were allocated. Lp(a) and Apo were analyzed in the baseline samples. Uni- and multivariable logistic regression analyses were used to estimate risks related to a 1 (ln) standard deviation increase in Lp(a) and the ratio of Apo B to Apo A1 (Apo B/A1 ratio). High levels of Lp(a) predicted surgery for AS in 203 patients with concomitant CAD (odds ratio [95% confidence intervals]) (1.29 [1.07-1.55]), but not in 132 patients without CAD (1.04 [0.83-1.29]) in the fully adjusted model. Similarly, a high Apo B/A1 ratio predicted surgery in patients with concomitant CAD (1.43 [1.16-1.76]) but not in those without CAD (0.87 [0.69-1.10]).

***Conclusions*—**High levels of Lp(a) and a high Apo B/A1 ratio were associated with surgery for AS in patients with concomitant CAD but not in those with isolated AS. This finding may lead to a new avenue of research for targeted risk factor interventions in this population. **(*J Am Heart Assoc*. 2017;6:e007160. DOI: 10.1161/JAHA.117.007160.)**

Key Words: aortic stenosis • aortic valve surgery • apolipoproteins • lipoprotein(a) • prospective cohort study • risk markers

In Western society, aortic stenosis (AS) is the most common valvular disease that requires surgery in adults. The prevalence of AS is between 2% and 4% in the population, and is higher in older age groups.[1] Currently, there is no medical therapy available to prevent the development or slow the progression of AS; hence, aortic valve replacement (AVR) is the only option in treating patients with symptomatic AS. AS is an obstruction of left ventricular outflow due to dysfunctional aortic valve leaflets, and its origin can be either congenital or acquired; acquired forms are typically classified as rheumatic or degenerative. Currently, degenerative valve calcification is considered the most common form of clinically significant AS. The etiology of degenerative calcification is unknown, but possible causes have been suggested, including the aging process, inflammatory mechanisms, and atherosclerosis. Atherosclerotic lesions and valvular calcification have similar histologies and similar associations with traditional cardiovascular risk factors. In fact, concomitant coronary artery disease (CAD) is a common finding in patients who require surgery for AS. Previously, the pathological process of valvular calcification was regarded as a passive degenerative process, but currently it is regarded as an active biological process, with histological evidence of inflammation and extracellular matrix remodeling that leads to bone formation. Hypercholesterolemia is associated with AS development,[2] but randomized trials that targeted a reduction in plasma cholesterol levels have failed to show any effect on progression rates,

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Clinical Perspective

What Is New?

- Aortic stenosis has several phenotypes related to valvular morphology and concomitant atherosclerosis.
- In this study we found that high plasma levels of lipoprotein (a) and a high apolipoprotein B/A1 ratio associated independently with future surgery for aortic stenosis.
- Notably, these associations were observed only in patients with concomitant coronary artery disease.

What Are the Clinical Implications?

- This finding may lead to a new avenue of research for targeted risk factor interventions in this population.

possibly due to advanced disease and unfavorable changes in the lipid profile.[3-5] However, the atherogenic profile may be better described with measurements of apolipoproteins and lipoprotein(a) [Lp(a)].[6,7]

Apolipoprotein B (Apo B) comprises the major fraction of protein content in low-density lipoprotein (LDL), and each particle harbors only 1 Apo B. Consequently, Apo B measurements reflect the number of particles, in contrast to LDL-cholesterol, which measures the amount of cholesterol in LDL-particles.[8] Clinically, at a given LDL-cholesterol concentration, the presence of a few, and thus larger, particles is more favorable than the presence of a large number of small particles. Apolipoprotein A1 (Apo A1) is the main apolipoprotein incorporated into high-density lipoprotein (HDL). Thus, the Apo B/Apo A1 ratio may be a better risk predictor of atherosclerotic disease than the LDL/HDL ratio.[9]

Another type of LDL particle, Lp(a), is also synthesized in the liver, and it contains apolipoprotein(a) [Apo(a)] in addition to Apo B.[7] Circulating levels of Lp(a) are mainly determined genetically; they are markedly stable over time in men, and they are usually not affected by diet or physical activity. In women, menopause is related to increasing levels of Lp(a) that have been related to increased risk for cardiovascular disease.[10] However, Lp(a) levels may vary considerably among individuals; ≈20% of Europids have elevated Lp(a) levels, that is, >50 mg/dL (≈100-125 nmol/L). The physiological role of Lp(a) is unclear, but high levels have been associated with ischemic atherosclerotic diseases such as myocardial infarction (MI) and stroke.[11] Lp(a) was recently associated with both the development of AS and its progression.[4,12-15] Indeed, prospective study designs and Mendelian randomization have suggested that Lp(a) may cause AS. However, those studies did not consider the potential influence of concomitant CAD.

In this prospective, case-referent study, we identified all patients who underwent surgery for AS and had baseline measurements available due to participation in 1 of 3 large, ongoing population-based studies in Northern Sweden. We aimed to examine baseline blood samples to determine whether circulating levels of Lp(a) and the Apo B/A1 ratio could predict future surgery for AS. We hypothesized that these associations might be affected by the concomitant presence of coronary arteriosclerosis.

Methods

Study Population

A total of 6691 patients underwent surgery for valvular heart disease and/or disease of the ascending aorta in the Department of Cardiothoracic Surgery, Umeå University Hospital, Umeå, Sweden, between March 1988 and December 2014. Before their first surgery, 708 of these patients had participated in 1 of 3 population-based health studies in the Northern Sweden Health and Disease Study, and they had donated blood for future research. Among these, 336 had later undergone surgery for AS. We retrieved plasma samples for each of these 336 patients, including 237 samples from the VIP (Västerbotten Intervention Programme), 37 samples from the MONICA (the Northern Sweden Monitoring of Trends and Determinants in Cardiovascular Disease) survey, and 62 samples from the MSP (Mammary Screening Program). VIP is an ongoing community intervention program with the aim of preventing cardiovascular disease and diabetes mellitus. In this program, all county residents at the ages of 30 (until 1995), 40, 50, and 60 years, were asked to participate in a health survey and to receive health counseling at their primary healthcare center.[16] MONICA enrollment involved asking randomly selected individuals to participate in a health survey. Participants were 25 to 74 years of age, and they resided in the counties of Västerbotten and Norrbotten.[17] The MSP cohort comprised women who attended routine mammography screenings.[18] Taken together, these 3 surveys included 140 414 participants up to December 2014, which reflected participation rates of 65% to 75%.

For each case we randomly selected 2 referents (controls) who were matched for sex, age (±2 years), type of survey (MONICA, VIP, or MSP), date of health survey (±4 months), and geographic area. We did not exclude referents or cases (patients) with a history of MI or cancer before the survey. In our cohort 3.3% of referents and 2.7% of cases had been diagnosed with cancer within 5 years before surgery (or the corresponding date for referents). Similarly, 1.3% of referents and 2.4% of cases reported a prior MI at survey.

The study protocol was approved by the Regional Ethics Review Board in Umeå, and it complied with the Declaration of

Helsinki. All participants provided written informed consent for future use of the data and blood samples.

Perioperative Characteristics

From hospital files, we acquired data on preoperative assessments, including the medical history, current medication, anthropometry, blood pressure, ECG, coronary angiogram, and echocardiography, when available. We also recorded perioperative details such as the nature of valvular disease (eg, malformations, calcification, and endocarditis), type of valvular intervention (ie, mechanical or biological prosthesis, or valvuloplasty), and the number of coronary grafts. According to established practice, most cases (99%) underwent a coronary angiogram, and any atheromatosis was taken to indicate CAD (found in 60% of all cases).

All 336 patients received an AVR to treat AS. In 84% of patients the primary indication was AS; the remaining 16% received aortic valvular surgery combined with another primary intervention, such as coronary artery bypass surgery (10%) or surgery for ascending aortic disease (5%).

Baseline Clinical Examinations and Biochemical Analysis

During the initial health survey, participants in VIP and MONICA were asked to complete a health questionnaire regarding their living conditions and cardiovascular risk factors. These participants also underwent anthropometry and blood pressure measurements. Participant weight was measured in light, indoor clothing without shoes, and recorded to the nearest 0.2 kg. Height was measured without shoes to the nearest centimeter. Body mass index (BMI) was calculated. Subjects were categorized by whether they had smoked tobacco (smokers, including current daily smokers and ex-smokers) or had never smoked tobacco (never-smokers).

An oral glucose tolerance test, with measurements of fasting and postload glucose levels, was performed routinely in the VIP, in 60% of MONICA participants, but not in the MSP. Diabetes mellitus was determined based on self-reported usage of antidiabetic medication, fasting plasma glucose levels ≥7.0 mmol/L, and/or postload plasma glucose levels ≥11.1 mmol/L (or ≥12.2 mmol/L based on capillary plasma measurements in the VIP). Impaired fasting glucose was defined as a fasting glucose level ≥6.1 and <7.0 mmol/L. Impaired glucose tolerance was defined as a postload glucose level ≥7.8 and <11.1 mmol/L (or ≥8.9 and <12.2 mmol/L in the VIP), combined with a nondiabetic fasting glucose level. The definition of glucose intolerance was impaired fasting glucose, impaired glucose tolerance, or diabetes mellitus.

In the MONICA and MSP surveys, blood pressure was recorded in the sitting position after 5 minutes of rest. Initially, a mercury sphygmomanometer was used, but from 2004, semiautomatic devices were used (Omron M7, Omron Corp, Kyoto, Japan). In the VIP survey, blood pressure was measured after 5 minutes of rest in the recumbent position until September 1, 2009; thereafter, it was measured in the sitting position with the devices described above. Measurements obtained with participants in the recumbent position were adjusted with a sex- and age-specific formula.[19] Hypertension was defined as a systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, and/or the use of antihypertensive medication.

Total serum cholesterol was measured at the time of the health survey with a benchtop analyzer (Reflotron®, Boehringer Mannheim GmbH Diagnostica, Mannheim, Germany) in the VIP until September 2009. After September 2009 in the VIP, and in the MONICA survey, serum cholesterol (mmol/L) was measured at a central laboratory with an enzymatic method (Boehringer Mannheim GmbH Diagnostica, Mannheim, Germany). Cholesterol values obtained with the benchtop method were adjusted to facilitate comparisons with the results measured at the central laboratory (total $\text{cholesterol}_{\text{Central lab}}=0.738+(0.901\times\text{total cholesterol}_{\text{Reflotron}})$.

Plasma samples were obtained after fasting for a minimum of 4 hours (extended to 8 hours after 1992). The samples were stored in a deep-freeze blood bank at −80°C until analysis.

In 2017, Apo A1, Apo B, and Lp(a) were analyzed on a Cobas® 8000 modular analyzer, c502 module. The reagents employed were Tina-quant apolipoprotein A1 and B (catalog Nos. 03032566122 and 03032574122, respectively, both version 2) and Tina-quant Lp(a) Generation 2 (catalog No. 05852625190; Roche Diagnostics, Basel Switzerland). For Lp(a), the lowest level of detection was 7 nmol/L. Apo A1 and Apo B were standardized to reference standards IFCC SP1-01 and SP3-07, respectively. Lp(a) was standardized to reference material IFCC SRM2B and was expressed in SI units (nmol/L). The Apo B/A1 ratio was calculated. To adjust measured Apo B levels for the amount of Apo B in the Lp(a) particle, we converted Apo B levels from grams per liter to nanomoles per liter and calculated the number of Apo B–containing particles not related to Lp(a), as described by Enkhmaa et al.[20] The adjusted ApoB level (in grams per liter) was used to calculate a Lp(a)-independent Apo B/A1 ratio. The total coefficients of variation were Apo A1 3.42% and 2.18% at levels of 0.86 and 1.45 mg/L, respectively; Apo B 1.93% and 2.19% at levels of 1.0 and 1.8 mg/L, respectively; and Lp(a) 2.4% and 3.2% at levels of 34 and 115 nmol/L, respectively.

Statistical Analysis

Continuous data were checked for normal distributions with formal tests and by visual inspection, and data were

transformed to the natural log (ln) scale when needed. The (ln) *z*-scores were calculated separately for men and women, and as a conservative approach, missing values were replaced with the median value obtained among the referents, calculated separately for men and women. The scores with replaced missing values were used in all models, thus using the entire data set. Continuous variables were also categorized into quartiles, based on the distribution of the referent values, and they were determined separately for men and women. Missing values were treated as a separate category and were not included in the tables.

Data are presented as mean [95% confidence interval]. Student t tests were used to analyze differences in the means between cases and referents. Associations between studied variables were tested with partial correlation analyses, adjusted for sex and age at the time of the survey. Within strata, the cases and referents had the same follow-up times in this nested, matched case-referent study. Therefore, we estimated odds ratios (OR) and 95% confidence intervals with logistic regression analyses (rather than Cox regression) and the conditional maximum likelihood routine designed for matched analysis. The influence of studied variables on future surgery for AS was tested in univariate and multivariable models. We used 2 models for multivariable analyses. The first model included Lp(a) and the Apo B/A1 ratio; the second model included the first model, with the addition of hypertension (yes/no), glucose intolerance (yes/no), and smoking (present or past/never). In a final model, BMI was added. The analyses were stratified for sex, age at surgery (less than 60 years or 60 years and more), the time between the survey and surgery (less than 5 years or 5 years and more), and the presence of any visible coronary arteriosclerosis on the preoperative angiogram. Finally, in separate analyses, we used the Lp(a)-independent ApoB/A1 ratio and excluded the MSP cohort. All calculations were performed with the statistical program SPSS version 24 (IBM, Armonk, NY).

Results

Patient Characteristics

Among the 336 patients with baseline measurements who underwent surgery for AS, 48% were women, and the mean age at surgery was 67.2 (66.3-68.2) years (Table 1). The median time (interquartile range) between the survey and surgery was 10.9 (9.3) years. The group of cases had a higher mean BMI, a higher mean blood pressure, a higher proportion of individuals with hypertension, and a higher proportion of individuals with glucose intolerance than the group of referents. The Apo B/A1 ratio was significantly higher among cases than among referents, particularly among women, among those who underwent surgery at ages above 60 years, and among those who underwent surgery more than 5 years after the health survey. Circulating levels of Lp(a) were also higher among cases than among referents, particularly among men, among cases who underwent surgery at ages above 60 years, and among those who underwent surgery more than 5 years after the health survey. The Apo B/A1 ratio and circulating levels of Lp(a) were markedly higher in cases who had concomitant CAD at surgery compared to referents. In contrast, cases without CAD did not have high Apo B/A1 ratios or Lp(a) levels compared to referents.

Correlations

In referents, a high Apo B/A1 ratio was associated with high BMI (r=0.25, P=0.001), high total cholesterol (r=0.52, P<0.001), and high systolic and diastolic blood pressures (r=0.10, P<0.05 and r=0.16, P<0.001, respectively). Levels of Apo B were not correlated with levels of Apo A1. After adjustments, the Apo B/A1 ratio remained associated with high BMI (r=0.24, P<0.001), high total cholesterol (r=0.53, P<0.001), and diastolic blood pressure (r=0.10, P<0.05). Similar results were seen if Lp(a) independent Apo B/A1 ratio was used or if only cases were analyzed (data not shown).

High Lp(a) was associated with high Apo B/A1 ratio in referents irrespective of adjustments (r=0.11, P=0.004 and r=0.12, P=0.002, respectively) but not in cases. High Lp(a) did not associate with Lp(a)-independent Apo B/A1 ratio in referents or in cases.

Univariable Analysis

In the univariable analysis, high levels of Lp(a), expressed as a 1-SD increase in ln Lp(a) levels, predicted surgery for AS (Table 2). In the categorical analysis, Lp(a) levels that corresponded to the highest quartile of Lp(a) (above 39 and 40 nmol/L in men and women, respectively) were associated with surgery. Similar patterns were observed after stratification by sex and in patients with CAD. In contrast, no association was found in patients without CAD.

The Apo B/A1 ratio, expressed as a 1-SD increase in the ln ratio, also predicted surgery for AS (Table 2). In the categorical analysis the upper 3 quartiles were associated with surgery, with a dose effect. Similar patterns were observed after stratification by sex and in patients with CAD. In contrast, no associations were observed in patients without CAD.

The associations between Lp(a) levels and the Apo B/A1 ratio and surgery for AS remained significant after excluding individuals who underwent surgery within 5 years after the survey. Separate univariate analyses of Apo B and Apo A1 and the risk for surgery for AS did not add more information (data not shown).

Table 1. Baseline Characteristics

	N (Referents/Cases)	Referents	Cases	*P* Value
Women, %	671/336	48.0	48.0	Matched
Age at survey, y	671/336	56.7 (56.0-57.3)	56.7 (55.8-57.6)	Matched
Age at surgery, y	. . ./336	. . .	67.2 (66.3-68.2)	
BMI, kg/m^2	655/322	26.1 (25.8-26.4)	26.9 (26.4-27.4)	0.01
Systolic blood pressure, mm Hg	545/270	135 (134-137)	138 (136-141)	0.04
Diastolic blood pressure, mm Hg	545/269	84 (84-85)	86 (85-87)	0.05
Hypertension, %	545/269	49.2 (45.0-53.4)	61.0 (55.1-66.8)	0.001
Glucose intolerance, %	490/242	19.8 (16.3-23.3)	26.4 (20.8-32.0)	0.05
Smoker, %	531/258	53.7 (49.4-57.9)	59.7 (53.7-65.7)	0.11
Total cholesterol, mmol/L	535/265	6.2 (6.1-6.3)	6.4 (6.2-6.5)	0.05
Apolipoprotein B, g/L†	647/310	1.09 (1.07-1.11)	1.13 (1.10-1.16)	0.05
Apolipoprotein A1, g/L†	647/309	1.41 (1.40-1.43)	1.40 (1.37-1.42)	0.25
Apolipoprotein B/A1 (ratio)†				
All	647/309	0.77 (0.76-0.79)	0.81 (0.79-0.84)	0.01
Men	335/155	0.82 (0.79-0.84)	0.85 (0.82-0.89)	0.12
Women	312/154	0.73 (0.71-0.75)	0.78 (0.74-0.82)	0.03
Age <60 y at surgery	138/69	0.73 (0.69-0.76)	0.75 (0.69-0.82)	0.48
Age ≥60 y at surgery	509/240	0.79 (0.77-0.81)	0.83 (0.81-0.86)	0.007
Surgery <5 y after survey	146/69	0.75 (0.72-0.79)	0.78 (0.72-0.84)	0.39
Surgery ≥5 y after survey	501/240	0.78 (0.76-0.80)	0.82 (0.80-0.85)	0.02
No CAD	256/127	0.74 (0.72-0.77)	0.73 (0.69-0.77)	0.47
CAD	389/181	0.79 (0.77-0.82)	0.88 (0.85-0.91)	<0.001
Lipoprotein(a), nmol/L†				
All	647/308	17.8 (16.2-19.6)	23.1 (19.8-27.0)	0.005
Men	335/154	16.4 (14.3-18.8)	22.0 (17.5-27.5)	0.03
Women	312/154	19.4 (17.0-22.2)	24.3 (19.6-30.2)	0.08
Age <60 y at surgery	138/69	15.2 (12.2-19.0)	15.4 (10.9-21.6)	0.96
Age ≥60 y at surgery	509/239	18.6 (16.7-20.6)	26.0 (21.9-30.9)	0.001
Surgery <5 y after survey	146/69	20.4 (16.5-25.2)	21.8 (15.4-30.9)	0.73
Surgery ≥5 y after survey	501/239	17.1 (15.4-19.0)	23.5 (19.8-28.0)	0.002
No CAD	256/127	17.0 (14.6-19.9)	18.0 (14.1-23.0)	0.69
CAD	389/180	18.4 (16.2-20.7)	27.5 (22.5-33.5)	0.001

BMI indicates body mass index; CAD, coronary artery disease; Glucose intolerance, impaired fasting glucose or impaired glucose intolerance or diabetes mellitus; Hypertension, systolic blood pressure ≥140 and/or diastolic blood pressure ≥90 and/or antihypertensive treatment; Smoker, present or previous smoker.
Values shown are numbers, means (†geometric), and proportions with 95% confidence intervals; *P*-values were based on the Student t test.

Multivariable Analysis

The predictive ability of Lp(a) remained significant after adjustment for the Apo B/A1 ratio and other traditional cardiovascular risk factors (OR 1.29 [1.07-1.55]) in patients with concomitant CAD (Table 3). After stratification by sex, similar point estimates were observed. The association between Lp(a) and surgery for AS was observed among patients aged 60 years or more at surgery and when the interval between the survey and surgery was longer than 5 years. In contrast, no association was observed among patients without CAD (OR 1.04 [0.83-1.29]), irrespective of sex, age at surgery, or interval between the survey and surgery. Furthermore, adjusting for BMI did not alter these associations (data not shown).

A high Apo B/A1 ratio independently predicted surgery for AS among patients with concomitant CAD (OR 1.43 [1.16-1.76]; Table 3). This association held irrespective of age at

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Table 2. Univariable Analysis

Patient Categories	Lp(a)					Apo B/A1 Ratio				
	Referents (N)	Cases (N)	All, OR (95% CI)	No CAD, OR (95% CI)	CAD, OR (95% CI)	Referents (N)	Cases (N)	All, OR (95% CI)	No CAD, OR (95% CI)	CAD, OR (95% CI)
z-score (missing)			1.22 (1.07-1.40)*	1.04 (0.85-1.28)	1.39 (1.15-1.67)*			1.23 (1.06-1.43)*	0.92 (0.74-1.15)	1.54 (1.26-1.90)*
z-score (missing replaced)			1.20 (1.05-1.37)*	1.04 (0.85-1.28)	1.33 (1.11-1.59)*			1.22 (1.06-1.41)*	0.92 (0.74-1.14)	1.51 (1.24–1.84)*
Quartiles										
Q1	164	78	1.00	1.00	1.00	162	52	1.00	1.00	1.00
Q2	161	55	0.77 (0.50-1.16)	0.42 (0.22-0.80)	1.29 (0.73-2.25)	162	81	1.59 (1.05-2.41)*	0.97 (0.55-1.69)	4.07 (1.89-8.77)*
Q3	161	60	0.81 (0.54-1.22)	0.63 (0.34-1.17)	1.10 (0.63-1.94)	161	80	1.69 (1.10-2.62)*	0.55 (0.28-1.05)	6.45 (2.94-14.15)*
Q4	161	115	1.54 (1.06-2.23)*	0.93 (0.53-1.64)	2.42 (1.43-4.08)*	162	96	1.91 (1.27-2.87)*	0.88 (0.48-1.62)	5.72 (2.71-12.05)*
Men			1.21 (1.01-1.45)*	1.00 (0.73-1.38)	1.31 (1.05-1.65)*			1.19 (0.97-1.46)	0.88 (0.63-1.24)	1.39 (1.07-1.81)*
Women			1.20 (0.99-1.45)	1.07 (0.82-1.40)	1.35 (1.02-1.79)*			1.25 (1.03-1.53)*	0.95 (0.71-1.26)	1.67 (1.23-2.27)*
Age <60 y at surgery			1.00 (0.77-1.30)	0.97 (0.71-1.33)	1.07 (0.65-1.75)			1.15 (0.85-1.56)	0.90 (0.62-1.31)	1.93 (1.03-3.61)*
Age ≥60 y at surgery			1.28 (1.10-1.49)*	1.10 (0.84-1.44)	1.37 (1.14-1.66)*			1.24 (1.06-1.46)*	0.93 (0.71-1.22)	1.46 (1.18-1.80)*
Surgery <5 y after survey			1.03 (0.79-1.35)	1.19 (0.82-1.73)	0.87 (0.59-1.27)			1.16 (0.87-1.57)	0.86 (0.57-1.29)	1.63 (1.03-2.59)*
Surgery ≥5 y after survey			1.26 (1.09-1.47)*	0.99 (0.77-1.26)	1.49 (1.22-1.82)*			1.24 (1.05-1.46)*	0.95 (0.73-1.23)	1.48 (1.19-1.84)*

Values are the odds ratios with (95% confidence intervals) for 1 (ln) SD increase (*z*-score) in the Lp(a) level or the Apo B/A1 ratio, as indicated. Asterisks indicate significant values. *z*-scores with missing values replaced were used for all uni- and multivariable calculations; 52 missing values for Lp(a) and 51 missing values for the Apo B/A1 ratio were replaced with the median values obtained for the referents (sex-specific). Cutoffs for the quartiles (Q1 to Q4, nmol/L) were: Lp(a) (men and women): 5.8, 13.3, 38.5 and 8.1, 14.9, 40.1, respectively; the Apo B/A1 ratios (men and women) were 0.67, 0.83, 1.01 and 0.60, 0.73, 0.88, respectively. *P*-values for indicating a trend: 0.015 for Lp(a) and 0.006 for the Apo B/A1 ratio. CAD indicates coronary artery disease CI, confidence interval.

Table 3. Multivariable Analysis

	Lp(a)		Apo B/A1 Ratio	
Patient Categories	Model 1	Model 2	Model 1	Model 2
All patients (N=336/671)				
All	1.17 (1.03-1.34)*	1.18 (1.03-1.35)*	1.19 (1.03-1.38)*	1.16 (1.00-1.35)*
Men	1.18 (0.98-1.42)	1.20 (0.99-1.45)	1.15 (0.93-1.42)	1.10 (0.89-1.37)
Women	1.17 (0.96-1.41)	1.16 (0.95-1.41)	1.23 (1.00-1.50)*	1.24 (1.00-1.53)*
Age <60 y at surgery	0.98 (0.75-1.28)	0.96 (0.72-1.27)	1.16 (0.85-1.57)	1.21 (0.87-1.68)
Age ≥60 y at surgery	1.25 (1.07-1.46)*	1.27 (1.08-1.49)*	1.20 (1.02-1.42)*	1.15 (0.97-1.36)
Surgery <5 y after survey	1.02 (0.78-1.34)	1.04 (0.79-1.38)	1.16 (0.86-1.57)	1.16 (0.86-1.57)
Surgery ≥5 y after survey	1.23 (1.05-1.43)*	1.26 (1.07-1.47)*	1.20 (1.01-1.41)*	1.15 (0.97-1.37)
Without CAD (N=132/264)				
All	1.06 (0.86-1.30)	1.04 (0.83-1.29)	0.91 (0.73-1.14)	0.87 (0.69-1.10)
Men	1.02 (0.74-1.41)	1.04 (0.74-1.47)	0.88 (0.62-1.24)	0.84 (0.58-1.22)
Women	1.09 (0.83-1.42)	1.02 (0.77-1.36)	0.93 (0.70-1.24)	0.88 (0.65-1.21)
Age <60 y at surgery	0.98 (0.72-1.35)	0.95 (0.68-1.33)	0.90 (0.62-1.32)	0.97 (0.65-1.47)
Age ≥60 y at surgery	1.12 (0.85-1.48)	1.09 (0.81-1.46)	0.91 (0.69-1.20)	0.82 (0.61-1.11)
Surgery <5 y after survey	1.20 (0.82-1.73)	1.23 (0.84-1.81)	0.85 (0.56-1.30)	0.89 (0.58-1.37)
Surgery ≥5 y after survey	1.00 (0.78-1.28)	0.98 (0.75-1.29)	0.95 (0.73-1.24)	0.87 (0.65-1.16)
With CAD (N=203/405)				
All	1.28 (1.07-1.53)*	1.29 (1.07-1.55)*	1.47 (1.20-1.79)*	1.43 (1.16-1.76)*
Men	1.26 (1.00-1.59)*	1.24 (0.98-1.58)	1.34 (1.02-1.75)*	1.30 (0.99-1.72)
Women	1.32 (0.99-1.76)	1.35 (1.00-1.83)*	1.65 (1.21-2.26)*	1.69 (1.22-2.35)*
Age <60 y at surgery	0.91 (0.53-1.56)	0.80 (0.41-1.55)	2.00 (1.01-3.94)*	3.04 (1.00-9.26)*
Age ≥60 y at surgery	1.33 (1.09-1.61)*	1.35 (1.11-1.64)*	1.42 (1.15-1.76)*	1.38 (1.10-1.72)*
Surgery <5 y after survey	0.76 (0.50-1.16)	0.85 (0.54-1.32)	1.76 (1.09-2.84)*	1.81 (1.10-2.98)*
Surgery ≥5 y after survey	1.44 (1.18-1.77)*	1.48 (1.20-1.83)*	1.43 (1.15-1.80)*	1.40 (1.11-1.78)*

Values are numbers (cases/referents), and the odds ratios (95% confidence intervals) for 1 (ln) SD increase (*z*-score) in the Lp(a) levels and the Apo B/A1 ratio, as indicated. Model 1 includes Lp(a) and the Apo B/A1 ratio; model 2 includes model 1 plus glucose intolerance (yes/no), hypertension (yes/no), and smoking (present or past/never). Asterisks indicate significant values. CAD indicates coronary artery disease.

surgery and the interval between the survey and surgery. Similar point estimates were observed in both men and women. In patients without CAD the Apo B/A1 ratio was not associated with surgery for AS (OR 0.87 [0.69-1.10]), irrespective of sex, age at surgery, or the interval between the survey and surgery. The association between a high Apo B/A1 ratio and surgery for AS remained significant after adjustment for BMI (data not shown).

Sensitivity Analysis

Using the Lp(a)-independent Apo B/A1 ratio in the uni- and multivariable models did not change the results (data not shown). After exclusion of the MSP cohort, the associations remained significant between high Lp(a) levels and AVR (OR 1.25 [1.02-1.53]) and between a high Apo B/A1 ratio and AVR (OR 1.38 [1.10-1.73]) among patients with concomitant CAD. No associations were observed among patients without any visible coronary atherosclerosis after exclusion of the MSP cohort (data not shown).

Discussion

In this nested, case-referent study, we showed that both high levels of Lp(a) and a high Apo B/A1 ratio were independently associated with future surgery for AS, but only in patients with concomitant CAD. Notably, Lp(a) was associated with surgery in older patients and in patients with a long observation period, a pattern not observed for the Apo B/A1 ratio.

Lp(a) and Risk of AS

Lp(a) is a lipoprotein that comprises an LDL particle and an Apo B molecule bound to an apolipoprotein(a) [Apo(a)]

molecule. Apo(a) shares characteristics with the plasminogen molecule.[7,21] The size of the Apo(a) particle varies markedly, depending on the number of kringle repeats, which is genetically determined; small isoforms are related to higher plasma levels due to faster release from hepatocytes. Several single-nucleotide polymorphisms (SNPs) within the *LPA* gene on chromosome 6 may also determine plasma levels of Lp(a). The rs10455872 and rs3798220 SNPs are both associated with small isoforms and high Lp(a) levels. It has been suggested that Lp(a) plays a causal role in the development of atherosclerotic diseases, and high circulating levels of Lp(a) were prospectively associated with coronary heart disease and ischemic stroke. In addition, genetically determined small isoforms of Lp(a), as a result of either kringle repeats or the above-mentioned SNPs, were associated with cardiovascular events; that is, Mendelian randomization studies have supported a causal relationship.[11,21-23] This concept was further supported by the finding that genetically determined low levels of Lp(a) due to loss of function of the proprotein convertase subtilisin/kexin type-9 protein were associated with reduced risks of both MI and AS.[24] The atherogenic properties of Lp(a) were demonstrated when Lp(a) was shown to entrap cholesterol in the intima by recruiting inflammatory cells and binding oxidized phospholipids; moreover, Lp(a) was also shown to interrupt fibrinolytic processes.[1,25]

Recently, several studies with different approaches have linked Lp(a) to AS in different populations. For example, Thanassoulis et al demonstrated that the SNP rs10455872 was associated with aortic valve calcification, determined with standard computed tomography scans, in different cohorts of individuals with various ethnic backgrounds.[14] In those studies the numbers of participants ranged from 745 to 3120, and 9% to 43% had detectable aortic valve calcium deposits. The levels of Lp(a) were strongly associated with the rs10455872 polymorphism, and they were also associated with aortic valve calcification. The presence of any concomitant coronary artery calcium deposition was taken into account. Furthermore, the same SNP could predict AVR in Swedish and Danish cohorts, but concomitant CAD was not reported.[14]

These findings have since been replicated in different populations and with different definitions of the outcome. Lp (a) levels were independently associated with aortic valve calcification but not with coronary artery calcification in a population of familial hypercholesterolemia.[26] Hospitalizations or deaths from AS, assessed according to the International Codes of Diseases codes, were predicted both by high Lp(a) levels and by the GG form of the SNP, rs10455872, in the EPIC-Norfolk cohort.[12] The largest study to date was the joint Copenhagen study, with 77 680 participants and 454 incident cases of AS, assessed according to the International Codes of Diseases and surgical codes.[13] Simultaneous coronary bypass procedures were not identified. Lp(a) levels at baseline were found to predict AS, irrespective of sex and previous MI, but only when incident cases with AVR were included. In the Copenhagen study Lp(a) levels were increased in carriers of the rs10455872 and rs3798220 alleles, and Lp(a) levels increased with decreasing numbers of kringle repeats. When combined, these genotypes explained 41% of the variation in plasma Lp(a) levels. In addition, carriers of the minor allele of rs10455872 showed increased risk of AS. Capoulade et al took another approach by studying the progression rate of known AS in a substudy of the ASTRONOMER (Aortic Stenosis Progression Observation: Measuring Effects of Rosuvastatin) trial.[4,5] They found that high levels of Lp(a) and oxidized phospholipids predicted rapid progression of AS. Of note, this association was observed, irrespective of the underlying valve pathology (ie, bicuspid), and it was more evident in younger patients (aged 57 years and under). Levels of Lp(a) and oxidized phospholipids increased substantially in patients randomized to statin treatment.

The present study added important information regarding Lp(a) and the risk of AS. The large size of the cohort and the careful validation of each case allowed us to stratify patients into clinically meaningful phenotypes. Our main finding was that the predictive abilities of Lp(a) and the Apo B/A1 ratio were restricted to patients with visible coronary atherosclerosis on an angiogram. This observation had not been reported previously, and it indicated that AS includes several phenotypes that should be considered in designing interventional trials. Moreover, this finding may be important for designing individualized therapies.

Our finding that Lp(a) and the Apo B/A1 ratio showed different patterns of cardiovascular risk was puzzling because genetically determined high Lp(a) levels represent a lifelong exposure, and cholesterol/apolipoprotein levels are related to lifestyle in adults. These results might be explained by unknown mechanisms that predisposed individuals to participate in a health survey or by features that contribute to AVR eligibility. In younger patients the bicuspid aortic valve represents a major underlying pathology. It is possible that the effects of Lp(a) are different between individuals with bicuspid and tricuspid valves. However, underlying pathology did not influence the Lp(a) effect on the progression rate.[4] Congenital malformations of the aortic valve were excluded in the Copenhagen study.[13] Nevertheless, only 17 cases of bicuspid valves were excluded in the latter study, which was a much lower rate than expected. That underrepresentation clearly indicated that the International Codes of Diseases were insufficient for phenotyping AS. Our study did not address this issue because we did not have data on valvular pathology collected at the time of the AVR.

Currently, no treatment option has been established for high Lp(a) levels, other than experimental and observational regimes.[27-33] Moreover, to date, no randomized controlled

trials have directly evaluated the effect of lowering Lp(a) on cardiovascular outcomes including AS development.

Lipids and Risk of AS

Several lipoproteins are associated with atherosclerotic cardiovascular disease. Previously, in a large cohort, Apo B and non-HDL cholesterol were strongly correlated with the presence of coronary calcium.[34] However, the Apo B/Apo A1 ratio may predict the risk of atherosclerotic disease better than the LDL/HDL ratio,[9] and a high Apo B/A1 ratio was strongly associated with MI in the INTERHEART case-control study.[35] High levels of total cholesterol and LDL have also been associated with the presence and development of AS.[36-39]

Based on this knowledge, 3 randomized placebo-controlled trials were performed with the aim of reducing progression of aortic valve stenosis with statins: the SALTIER (Scottish Aortic Stenosis and Lipid Lowering Trial, Impact on Regression), the SEAS (Simvastatin and Ezetimibe in Aortic Stenosis), and the ASTRONOMER trials. In the SALTIER trial, 155 patients with asymptomatic aortic valve calcium deposits were followed for 25 months.[40] LDL levels were lowered by 53% in the treatment arm, but no differences in aortic-jet velocity were identified. In the SEAS trial, 1873 patients with mild to moderate asymptomatic stenosis were followed for 52 months.[3] The primary outcome was a composite of major cardiovascular events including AVR. LDL levels were reduced by 54% in the treatment arm, but neither a change in peak aortic-jet velocity nor the incidence of AVR differed between arms. However, the incidence of ischemic cardiovascular events was lower in the active treatment arm. Notably, levels of Apo B were significantly lower in the treatment arm,[41] but Apo B levels did not predict AS in another study.[13] In the ASTRONOMER trial 269 patients with mild to moderate AS were followed for 3.5 years.[5] LDL-cholesterol was reduced by 55%, but no effects were observed on progression rate. Lp(a) levels were not reported in the SEAS or the SALTIER trials.

The development of AS is a complex process involving several pathways, including lipid deposition, inflammation, and calcification.[1] Once these processes are initiated, statins and LDL-lowering treatments may not affect the process; however, most trials have implemented relatively short treatment periods. A potentially positive effect of statins may be counteracted by an increase in Lp(a) levels.[42] Furthermore, clinical trials typically include patients who fulfill specific criteria; thus, the results of those studies may not be generalizable to patients with multiple comorbidities and risk factors for AS.

Limitations and Strengths

Our study had several limitations. First, features of the study population may have limited the generalizability of our findings. The population had a Europid background rooted in the northern part of Sweden, and circulating levels of Lp(a) have been shown to differ among different ethnicities.[7,20] In addition, the inclusion criteria for the VIP survey may have affected the age distribution of the patient population; they only included individuals who had participated in the health survey at ages 30, 40, 50, or 60 years. This may have led to an underrepresentation of younger patients. Moreover, the study design did not permit a detailed description of the underlying morphology, that is, a bicuspid versus a tricuspid aortic valve. However, in patients younger than 60 years of age, bicuspid valves are by far the more common valve morphology. In future studies, valve morphology should be carefully determined enabling further evaluation of the different phenotypes and the impact of risk markers such as lipids and Lp(a). Finally, this study only included patients who met indications for surgery; thus, we excluded patients with a severe comorbidity that caused a contraindication for surgery as well as patients with only slightly affected aortic valves.

A second limitation of our study concerned methodology. The subset of patients without CAD was defined by the absence of any visible atherosclerosis, but we could not exclude any atherosclerotic changes in the vessel wall that might not have affected the vessel lumen. Apparently, this issue did not present a large problem, however, because clear differences in predictive ability were observed between CAD and non-CAD groups. Furthermore, the presence of CAD was analyzed as a categorical value (yes/no) without taking into account the extent of atherosclerosis. The matched design precludes us from studying the impact of matched factors on the risk for future surgery, and it is not possible to test if the risk related to Lp(a) and Apo B/A1 ratio differs between men and women, except for showing that the point estimates were similar after stratification for sex (see Table 2).

A strength of our study is that we analyzed lipoprotein parameters insensitive to fasting status, that is, Apo B, Apo A1, and Lp(a), as the other parameters (with the exception of HDL-cholesterol) are influenced by fasting status.[43] We do not have data on exact fasting time in the VIP and MONICA cohorts, and the participants in the MSP cohort were not fasting. If there were any (unlikely) effect of fasting status on Lp(a) and Apo B and A1 levels, this should affect cases and referents equally and attenuate the results. Similarly, we do not have data on menopausal status or statin usage, which could affect levels of Lp(a) and Apo B and A1.[10] However, because age is a matching criterion, a woman in menopause is most probably matched with female referents in menopause. The usage of statins at the time of survey was probably very low, as the majority were surveyed before year 2000 (89%), and 98% did not report any MI before the survey.

Our population is mainly Europid, and the ethnic contribution to the variability in Lp(a) levels should be low.[7,20] Still, the results were very similar using a Lp(a)-independent Apo B/A1

ratio. This correction should, however, be done in other populations with other/unknown ethnic mixes. Furthermore, our samples were analyzed with the preferred method for determining Lp(a) levels, in the same laboratory on 1 occasion. Our assay measured the molar concentrations of Lp(a), traceable to the World Health Organization/International Federation of Clinical Chemistry reference material, and the results were not affected by the size of the isoforms. In contrast, previous studies typically determined the mass concentration (mg/dL) of plasma Lp(a) levels.[44] That method does not measure the different Lp(a) isoforms equally, and thus, the measurements are not traceable to international standards. Consequently, those values cannot be accurately interconverted.

Finally, the stability of Lp(a) at −70°C has been evaluated.[45] An instability was noted for cases with cardiovascular disease but not for controls. This instability resulted in lower values (−23% at levels 43-345 mg/L), but this change was independent of isoform size. Even if the instability also applies for patients with aortic stenosis, this should attenuate differences between the cases and the referents. Still, we found a significant association for Lp(a) in relation to surgery for aortic stenosis.

From an analytical point of view, by measuring Lp(a) independent of number of kringles (isoform size) using a method standardized to molar concentration, we expect our method to be less susceptible to preanalytical instability compared to previous methods for quantitating Lp(a), which were influenced by number of kringle repeats.

Conclusion

In this study we found that high plasma levels of Lp(a) and a high Apo B/A1 ratio were associated with future surgery for AS, independent of traditional risk factors such as hypertension, glucose intolerance, and smoking. Notably, these associations were only observed in patients with concomitant CAD. This finding has not been demonstrated previously, and it suggested that patients with AS have different phenotypes. These results may open a new avenue of research on targeted risk factor interventions in this population.

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Author Contributions

Söderberg and Johansson designed the study, Ljungberg evaluated patient data, and Hultdin performed and interpreted the biochemical analyses. Bergdahl and Norberg contributed to aspects related to the cohort design. Ljungberg and Holmgren drafted the manuscript, and all authors revised the manuscript critically for important intellectual content. All authors approved the final version and are accountable for all aspects of the manuscript.

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Disclosures

None.

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SUPPLEMENTAL MATERIAL

Table S1 Among those in the CARDIA Brain MRI sub-study, differences in the analytical sample between individuals with and without available measurements of left ventricular ejection fraction and left atrial volume

Characteristic	LVEF			LAV		
	Known (n=297)	Missing (n=351)	p value	Known (n=406)	Missing (n=242)	p value
Age, years	50.3±3.5	50.5±3.4	0.34	50.3±3.6	50.7±3.3	0.13
Education, years	14.9±2.3	15.2±2.3	0.18	15.1±2.4	15.0±2.2	0.84
Women, n (%)	144 (48.5)	195 (55.6)	0.07	209 (51.5)	130 (53.7)	0.58
African Americans, n (%)	126 (42.4)	118 (33.6)	0.02	166 (40.9)	78 (32.2)	0.03
SBP, mm Hg	116.9±13.1	117.5±15.3	0.60	117.3±14.3	117.1±14.4	0.89
BMI	28.4±5.5	29.0±5.9	0.16	28.5±5.6	29.1±5.8	0.15
Sedentary time, hours/day	6.6±4.1	7.0±3.9	0.29	6.7±3.8	7.0±4.2	0.42

LVEF, left ventricular ejection fraction; LAV, left atrial volume; SBP, systolic blood pressure; BMI, body mass index. Data are presented as means ± standard deviations or n (%). All data come from year 25. Independent sample t-test was used to find differences between groups for continuous variables and χ^2 test for binary variables

Table S2 Differences between the study sample and other participants at year 25: CARDIA Brain MRI sub-study

	MRI study sample (n=648)	Other participants (n=2851)	p value
Age, years	50.4±3.5	50.1±3.7	0.4
Education, years	15.0±2.3	15.0±2.6	0.9
Women, n (%)	339 (52)	1641 (58)	0.05
African Americans, n (%)	244 (38)	1396 (49)	<0.001
SBP, mm Hg	117.2±14.3	119.0±15.8	0.01
DBP, mm Hg	72.7±10.6	74.2±11.0	0.01
BMI	28.7±5.7	30.5±7.5	<0.001
Smokers, n (%)	95 (15)	494 (17)	0.02
Sedentary time, hours/day	6.8±4.0	7.4±4.5	0.01

SBP, systolic blood pressure; BMI, body mass index. Data are presented as means ± standard deviations or n (%). Smokers are defined as current smokers. All data come from year 25. Independent sample t-test was used to find differences between groups for continuous variables and χ^2 test for binary variables

Table S3 Differences between Caucasians vs. African Americans and men vs. women: CARDIA Brain MRI sub-study

Characteristic	Caucasians (n=404)	African Americans (n=244)	p value	Men (n=309)	Women (n=339)	p value
Age, years	51.0±3.3	49.4±3.5	<0.001	50.3±3.4	50.5±3.5	0.41
Education, years	15.6±2.2	14.1±2.1	<0.001	14.8±2.2	15.3±2.3	0.01
SBP, mm Hg	113.8±12.5	122.9±15.5	<0.001	119.4±12.5	115.2±15.6	<0.001
BMI	27.7±5.4	30.4±5.9	<0.001	28.4±4.6	29.0±6.6	0.13
Smokers, n (%)	45 (11)	50 (21)	<0.001	50 (16.2)	45 (13.3)	0.32
Sedentary time	5.8±2.9	8.4±4.9	<0.001	7.1±4.2	6.6±3.8	0.13

SBP, systolic blood pressure; BMI, body mass index. Data are presented as means ± standard deviations or n (%). All data come from year 25. Smokers are defined as current smokers. Independent sample t-test was used to find differences between groups for continuous variables and χ^2 test for binary variables

Table S4 Associations of cardiac parameters with brain volumes: CARDIA Brain MRI sub-study

Cardiac parameter, per SD	ß (95% CI)		
	Gray matter	White matter	Total brain
LVEF (n=297)	-0.763 (-3.103; 1.577)	1.512 (-1.285; 4.309)	0.749 (-2.582; 4.079)
LAV (n=406)	-0.042 (-2.187; 2.103)	-1.915 (-4.444; 0.615)	-1.957 (-4.952; 1.039)
LV mass (n=627)	0.892 (-1.016; 2.800)	-0.027 (-2.147; 2.093)	0.864 (-1.647; 3.376)

	Abnormal white matter volume					
	None		Little (≤ 0.3 cm^3)		High (> 0.3 cm^3)	
	n		n	OR (95% CI)	n	OR (95% CI)
LVEF	62	Reference	120	0.94 (0.68; 1.30)	115	1.04 (0.74; 1.46)
LAV	82		164	0.73 (0.54; 0.97)	160	0.92 (0.69; 1.22)
LV mass	122		236	1.18 (0.92; 1.52)	269	1.15 (0.90; 1.49)

LVEF, left ventricular ejection fraction; LAV, left atrial volume; LV mass, left ventricular mass; CI, confidence interval; SD, standard deviation; OR, odds ratio.

ß with 95% CI is derived from linear regression and is the coefficient for an association of the cardiac parameter (per standard deviation) with the measure of brain volume. OR with 95% CI is derived from multinomial logistic regression for an association of the cardiac parameter with high and little abnormal white matter volume, when compare to no abnormal white matter.

The models are adjusted for age, sex, race/ethnicity, field center, years of education and intracranial volume. Each cardiac parameter was transformed into Z score and entered into each model separately.