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Original Article

# Lipoprotein(a), Oxidized Phospholipids, and Aortic Valve Microcalcification Assessed by <sup>18</sup>F-Sodium Fluoride Positron Emission Tomography and Computed Tomography

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## ABSTRACT

**Background:** Lipoprotein(a) (Lp[a]) is the preferential lipoprotein carrier of oxidized phospholipids (OxPLs) and a well-established genetic risk factor for calcific aortic valve stenosis (CAVS). Whether Lp(a) predicts aortic valve microcalcification in individuals without CAVS is unknown. Our objective was to estimate the prevalence of elevated Lp(a) and OxPL levels in patients with CAVS and to determine if individuals with elevated Lp(a) but without CAVS have higher aortic valve microcalcification.

## RÉSUMÉ

**Contexte :** La lipoprotéine(a) (Lp[a]), la principale lipoprotéine assurant le transport des phospholipides oxydés (PLOx), est un facteur de risque génétique bien établi de la sténose aortique calcifiante (SAC). On ignore si la présence de Lp(a) est un facteur prédictif de la microcalcification de la valve aortique chez les individus non atteints de SAC. Notre objectif était d'estimer la prévalence de taux élevés de Lp(a) et de PLOx chez des patients atteints de SAC et de déterminer si la microcalcification de la valve aortique est plus marquée chez les

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\*These authors contributed equally to the content of this manuscript.

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See page 139 for disclosure information.

Calcific aortic valve stenosis (CAVS) is the most common form of valvular heart disease, and its prevalence is steadily increasing in high-income countries, affecting approximately 3% of the population aged more than 65 years.<sup>1</sup> The only effective treatment for CAVS is aortic valve replacement (AVR). Genetic variations at the *LPA* locus on chromosome 6 are associated with elevated plasma lipoprotein(a) [Lp(a)]

**Methods:** We recruited 214 patients with CAVS from Montreal and 174 patients with CAVS and 108 controls from Québec City, Canada. In a second group of individuals with high ( $\geq 75$  nmol/L,  $n = 27$ ) or low ( $< 75$  nmol/L,  $n = 28$ ) Lp(a) levels, 18F-sodium fluoride positron emission tomography/computed tomography was performed to determine the difference in mean tissue-to-background ratio (TBR) of the aortic valve.

**Results:** Patients with CAVS had 62.0% higher Lp(a) (median = 28.7, interquartile range [8.2-116.6] vs 10.9 [3.6-28.8] nmol/L,  $P < 0.0001$ ), 50% higher OxPL-apolipoprotein-B (2.2 [1.3-6.0] vs 1.1 [0.7-2.6] nmol/L,  $P < 0.0001$ ), and 69.9% higher OxPL-apolipoprotein(a) (7.3 [1.8-28.4] vs 2.2 [0.8-8.4] nmol/L,  $P < 0.0001$ ) levels compared with individuals without CAVS (all  $P < 0.0001$ ). Individuals without CAVS but elevated Lp(a) had 40% higher mean TBR compared with individuals with low Lp(a) levels (mean TBR =  $1.25 \pm 0.23$  vs  $1.15 \pm 0.11$ ,  $P = 0.02$ ).

**Conclusions:** Elevated Lp(a) and OxPL levels are associated with prevalent CAVS in patients studied in an echocardiography laboratory setting. In individuals with elevated Lp(a), evidence of aortic valve microcalcification by 18F-sodium fluoride positron emission tomography/computed tomography is present before the development of clinically manifested CAVS.

levels and the risk of aortic valve calcification (AVC) accumulation and CAVS in the general population.<sup>2-4</sup> It has been hypothesized that oxidized phospholipids (OxPLs) transported by apolipoprotein (apo)-B-containing lipoproteins (OxPL-apoB and OxPL-apo[a]) are pathologically implicated.<sup>5-8</sup> Although Lp(a) and OxPL levels are associated with CAVS risk in the general population,<sup>9,10</sup> whether Lp(a) and OxPL levels are associated with CAVS presence or severity in patients with established CAVS followed clinically in echocardiography laboratories without AVR is currently unknown. Although genetic variations at the *LPA* locus have been linked with macroscopic AVC deposits on computed tomography (CT) and Lp(a) levels linked with AVC accumulation in patients with familial hypercholesterolemia,<sup>2,11</sup> whether elevated Lp(a) or OxPL levels may be associated with early developing microcalcification in patients without CAVS is unknown.

Studies have shown that CT is a highly sensitive technique for the assessment of established macroscopic deposits of AVC. However, CT does not quantify early valve calcification (often referred to as “microcalcification”).<sup>12</sup> Positron emission tomography (PET)-CT imaging is accurate and reproducible to detect and quantify inflammation (18F-fluorodeoxyglucose uptake) and developing microcalcification activity (18F-sodium fluoride [NaF] uptake) into aortic valve hydroxyapatite,<sup>13-16</sup> and the measurement of valvular 18F-NaF uptake by PET/CT provides a novel biomarker of early disease activity and progression. This new imaging biomarker provides important incremental value to optimally study the association between Lp(a) and OxPL on CAVS pathobiology. Whether individuals with high Lp(a) levels are characterized by higher aortic valve microcalcification is currently unknown.

individus affichant des taux élevés de Lp(a) en l'absence de SAC.

**Méthodologie :** Nous avons recruté 214 patients atteints de SAC à Montréal et 174 patients atteints de SAC et 108 patients témoins à Québec (Canada). Dans un second groupe de patients présentant des taux de Lp(a) élevés ( $\geq 75$  nmol/l,  $n = 27$ ) ou faibles ( $< 75$  nmol/l,  $n = 28$ ), une tomographie par émission de positons au fluorure de sodium marqué au <sup>18</sup>F a été réalisée en vue de comparer la valeur moyenne du rapport signal/bruit (RSB) de la valve aortique.

**Résultats :** Les patients atteints de SAC présentaient des taux de Lp(a) plus élevés de 62,0 % (médiane = 28,7, intervalle interquartile [de 8,2 à 116,6] vs 10,9 [de 3,6 à 28,8] nmol/l,  $p < 0,0001$ ), des taux de OxPL-apolipoprotéine-B plus élevés de 50 % (2,2 [de 1,3 à 6,0] vs 1,1 [de 0,7 à 2,6] nmol/l,  $p < 0,0001$ ) et des taux de PLOx-apolipoprotéine(a) plus élevés de 69,9 % (7,3 [de 1,8 à 28,4] vs 2,2 [de 0,8 à 8,4] nmol/l,  $p < 0,0001$ ) comparativement aux patients non atteints de SAC (toutes les valeurs  $p < 0,0001$ ). Les patients non atteints de SAC mais présentant des taux élevés de Lp(a) avaient un RSB moyen supérieur de 40 % à celui des individus affichant un faible taux de Lp(a) (RSB moyen =  $1,25 \pm 0,23$  vs  $1,15 \pm 0,11$ ,  $p = 0,02$ ).

**Conclusions :** Des taux élevés de Lp(a) et de PLOx sont associés à la prévalence de la SAC chez des patients étudiés par échocardiographie. Chez les individus présentant un taux élevé de Lp(a), les signes d'une microcalcification de la valve aortique, décelés par tomographie par émission de positons au fluorure de sodium marqué au <sup>18</sup>F /tomodensitométrie sont présents avant l'apparition des manifestations cliniques de la SAC.

Our first objective was to determine whether patients with established CAVS actively followed clinically by echocardiography, but without AVR, had higher Lp(a), OxPL-apoB, and OxPL-apo(a) levels compared with individuals without CAVS. Our second objective was to determine whether Lp(a), OxPL-apoB, and OxPL-apo(a) were associated with CAVS severity as assessed by echocardiography. Third, we sought to determine, in individuals without CAVS, whether those with elevated Lp(a) levels were characterized by aortic valve microcalcification assessed by 18F-NaF PET/CT. Finally, to inform the future development of Lp(a) and OxPL-lowering therapies, we sought to identify the number of patients with CAVS with high Lp(a) above certain thresholds in contemporary clinical settings.

## Methods

### Study participants

A series of consecutive patients with mild to severe CAVS who did not undergo AVR were recruited at the echocardiography laboratory at the Québec Heart and Lung Institute (QHLI) and the McGill University Health Center. Exclusion criteria included the presence of mitral valve stenosis, mitral insufficiency (moderate or more), aortic insufficiency (moderate or more), and heart failure (ejection fraction  $< 40\%$ ). Patients were excluded if they had CAVS of rheumatic etiology or any type of cancer that required radiotherapy in the thoracic area (breast, trachea, bronchus, or lung cancer) before the diagnosis of CAVS. Women were also excluded if they were pregnant or lactating. At the QHLI, monthly interrupted

**Table 1.** Clinical characteristics of patients with versus without CAVS

	Controls		Patients with CAVS	
	(Quebec cohort) (n = 108)		Quebec cohort (n = 174)	Montreal cohort (n = 214)
Clinical characteristics				
Age, y	62.4 ± 7.4		65.3 ± 12.3*	73.6 ± 12.4
Male, n (%)	57 (53)		117 (67)*	119 (56)
Body mass index, kg/m <sup>2</sup>	28.1 ± 5.0		29.6 ± 5.8*	27.7 ± 6.8
Waist circumference, cm	98.6 ± 13.1		103.5 ± 18.2*	NA
Systolic blood pressure, mm Hg	130.0 ± 15.5		132.2 ± 17.2	129.6 ± 22.3
Diastolic blood pressure, mm Hg	79.4 ± 10.8		75.5 ± 9.4*	68.4 ± 11.8
Heart rate, beats/min	69.9 ± 11.9		66.9 ± 12.8	74.9 ± 13.8
Diabetes, n (%)	16 (15)		46 (26)*	67 (31)
CAD, n (%)	12 (11)		58 (33)*	89 (42)
Hypertension, n (%)	36 (34)		123 (71)*	146 (68)
Smoking (past or current), n (%)	56 (52)		122 (70)*	107 (52)
Hyperlipidemia, n (%)	45 (42)		125 (72)*	151 (71)
Doppler echocardiographic data				
Mean gradient, mm Hg	4.2 ± 1.5		17.3 ± 9.4*	36.0 ± 21.4
Peak gradient, mm Hg	7.7 ± 2.5		31.5 ± 15.6*	NA
Peak aortic jet velocity, cm/s	137.5 ± 21.7		274.6 ± 59.1*	372.6 ± 110.5
Indexed aortic valve area, cm <sup>2</sup> /m <sup>2</sup>	1.3 ± 0.3		0.7 ± 0.2*	0.5 ± 0.2
Laboratory data				
Total cholesterol, mmol/L	4.8 ± 1.1		4.3 ± 1.1*	3.9 ± 1.2
Triglycerides, mmol/L	1.9 ± 0.9		1.6 ± 0.9*	1.4 ± 0.7
HDL-C, mmol/L	1.4 ± 0.4		1.4 ± 0.4	1.2 ± 0.4
LDL-C, mmol/L	2.6 ± 1.0		2.2 ± 0.9*	2.1 ± 1.0

CAD, coronary artery disease; CAVS, calcific aortic valve stenosis; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NA, not available.

Data are presented as mean ± standard deviation or n (%).

\*Significantly different from controls (Quebec cohort,  $P < 0.05$ ).

time series were performed to recruit individuals without CAVS undergoing Doppler echocardiography (controls with normal aortic valves). Additional controls were also recruited through the echocardiography laboratory and advertisement at the QHLI. Each participant with or without CAVS completed detailed questionnaires about their health status, medication, cardiovascular history, family history, and ethnic origins. Previous and current medical history included history of smoking, documented diagnoses of hypertension (patients receiving antihypertensive medications or having known but untreated hypertension [blood pressure  $\geq 140/90$  mm Hg]), diabetes (fasting glucose  $\geq 7$  mmol/L or treatment with antidiabetic medication), and detailed information on current medication was collected. Body weight, height, and waist circumference were measured following standardized procedures. Blood pressure and heart rates were also assessed. Peak aortic jet velocity, aortic valve area, peak and mean transvalvular gradients, and aortic valve morphology were measured by Doppler echocardiography.<sup>17,18</sup> Aortic valve calcium burden was semiquantitatively assessed on echocardiography by cardiologists blinded to patients' Lp(a) levels and categorized as mild, moderate, or severe according to the criteria proposed by Rosenhek et al.<sup>19</sup> The study protocols were approved by the Ethics Committees of the 2 institutions, and all patients signed a written informed consent.

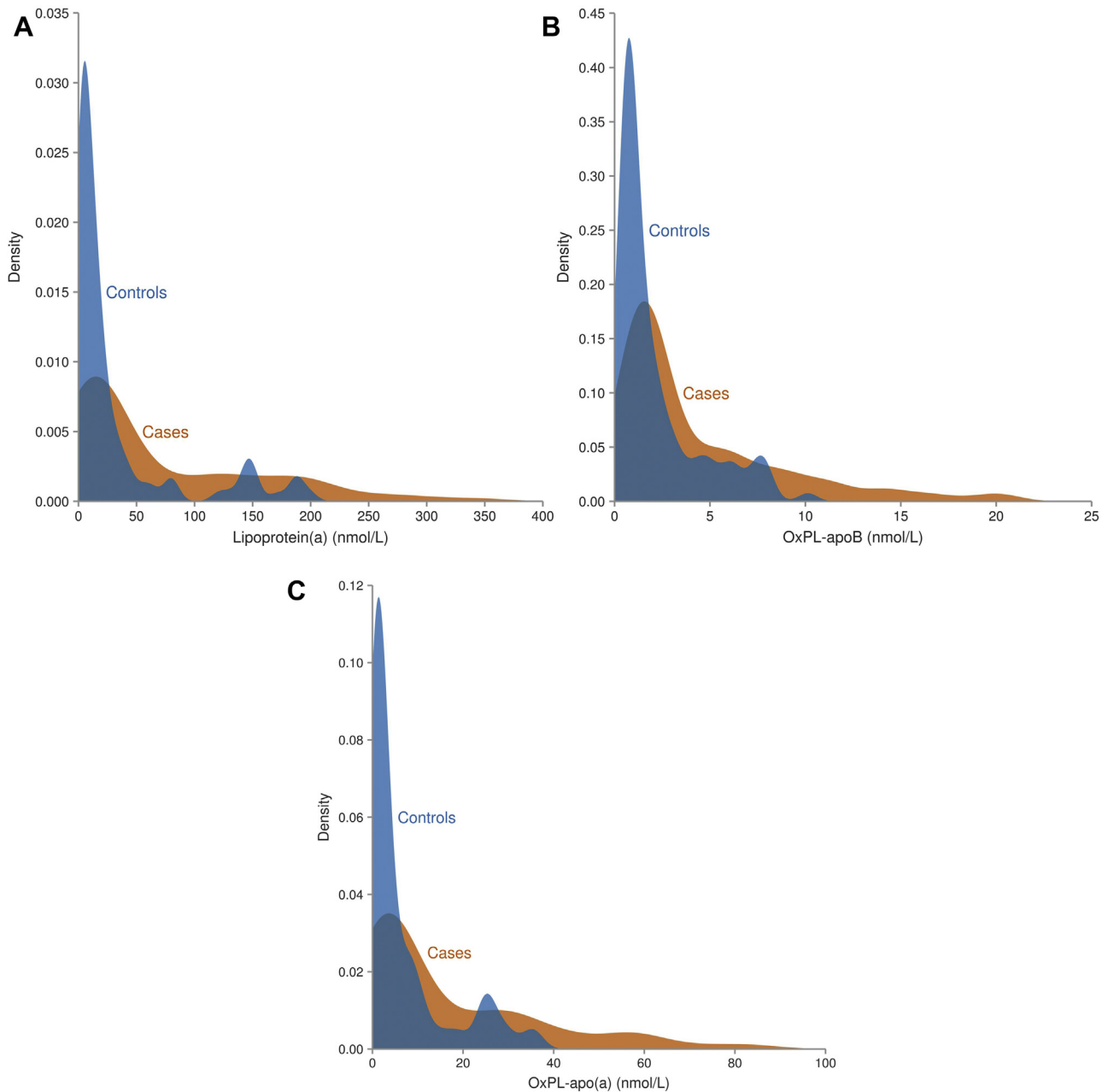
#### Assessment of aortic valve microcalcification by 18F-NaF PET/CT and macrocalcification by CT

Aortic valve microcalcification was assessed in a second cohort of participations without CAVS. All participants who underwent 18F-NaF PET/CT had plasma creatinine levels  $> 30$   $\mu\text{mol/L}$  (assessed  $< 2$  weeks before PET/CT). 18F-NaF

PET and CT were performed on integrated PET/CT scanners (GE Discovery RX16; GE Healthcare, Chicago, IL). Doses of 125 MBq of 18F-NaF were injected intravenously. PET scanning started after a 60-minute uptake period for a 30-minute bedtime. A CT scan for attenuation correction was first performed (low-dose 120 kV, 10 mA). Then, 2 CT scans (low-dose 120 kV, 150 mA) were performed centered over the aortic valve, one without contrast followed by a second one after injection of 70 mL of isomolar contrast medium. Analysis of AVC and NaF uptake was performed offline on a dedicated platform (OsiriX MD, Pixmeo SARL, Bernex, Switzerland). AVC was measured on noncontrast electrocardiogram-gated breath-hold CT scans and expressed in Agatston units. PET and contrast CT images were reoriented into the plane of the aortic valve, and circular regions of interest were drawn on adjacent 3-mm slices from the ascending aorta to the left ventricular outflow tract to assess the entire valve.<sup>20</sup> The mean and maximum standard uptake values were calculated for each slice. The 2 highest scores from contiguous slices were averaged and corrected for blood pool activity (right atrium) to provide mean tissue-to-background ratios (TBRs). These images were analyzed by an investigator blinded to all clinical and biomarker data.

#### Laboratory measurements

Nonfasting plasma samples were collected in ethylenediaminetetraacetic acid, aliquoted into microtubes, and stored at  $-80^\circ\text{C}$  until analysis. Plasma total cholesterol, triglyceride, high-density lipoprotein cholesterol, and glucose were measured using colorimetric enzymatic assays (Roche Diagnostics, Indianapolis, IN). Low-density lipoprotein cholesterol concentration was calculated using the Friedewald



**Figure 1.** Distribution and lipoprotein(a) (Lp[a]) (A), oxidized phospholipid (OxPL)-apolipoprotein (apo)B (B), and OxPL-apo(a) (C) levels in patients with versus without calcific aortic valve stenosis (CAVS) of the Quebec cohort.

formula. Plasma Lp(a) levels were measured by turbidimetric assay using the Tina-quant Lipoprotein(a) Gen.2 system (Cobas integra 400/800, Roche Diagnostics, Mannheim,

**Table 2.** Correlations among Lp(a), OxPL-apoB, and OxPL-apo(a) with aortic valve disease severity parameters in patients with CAVS of the Quebec cohort

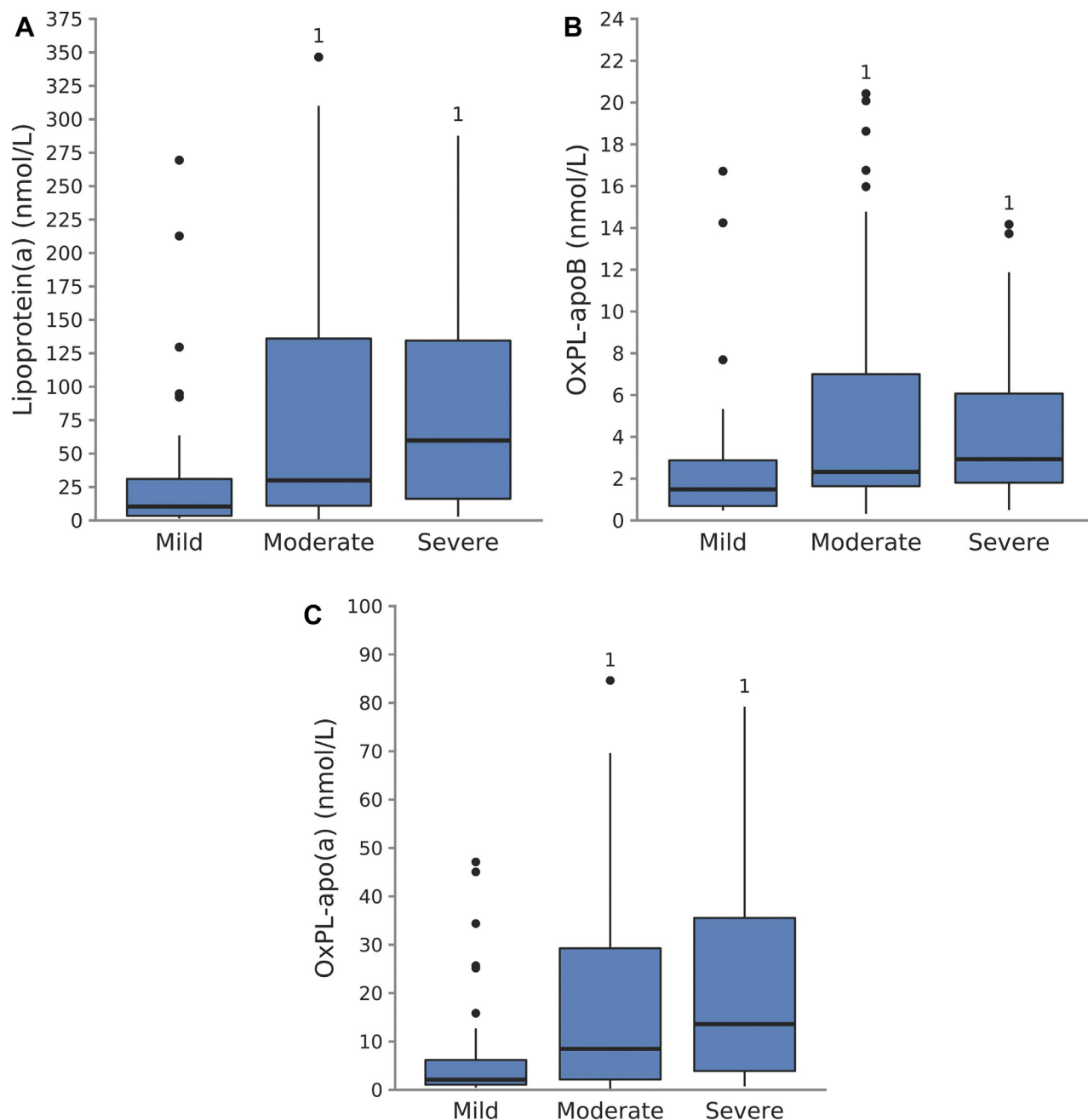
	Lp(a)	OxPL-ApoB	OxPL-Apo(a)
Peak aortic jet velocity, cm/s	-0.02 (0.8)	0.07 (0.4)	-0.03 (0.7)
Peak gradient, mm Hg	-0.02 (0.8)	0.07 (0.4)	-0.03 (0.7)
Mean gradient, mm Hg	0.02 (0.8)	0.08 (0.4)	0.008 (0.9)
Indexed aortic valve area, cm <sup>2</sup> /m <sup>2</sup>	-0.02 (0.8)	-0.06 (0.5)	-0.01 (0.8)

Apo, apolipoprotein; Lp(a), lipoprotein(a); OxPL, oxidized phospholipids. Data are presented as  $r$  ( $P$  value).

Germany) and by a chemiluminescent immunoassay developed by Tsimikas et al.<sup>21</sup> OxPL-apoB and OxPL-apo(a) were measured with chemiluminescent immunoassays as previously described.<sup>22</sup> The assay methodology is unchanged, but the calibrators were modified to allow reporting of units in molar concentration (nmol/L).

### Statistical analyses

Unpaired Student  $t$  tests and chi-square tests were respectively used to assess differences between continuous (on log-transformed values) and categorical clinical variables in patients with versus without CAVS. Spearman correlation coefficients and 1-way analyses of variance were performed to assess the relationship between CAVS severity and Lp(a) and



**Figure 2.** Lp(a) (A), OxPL-apoB (B), and OxPL-apo(a) (C) levels in patients with CAVS separated on the basis of estimated aortic valve calcium accumulation by echocardiography. 1: Statistically different than the group with mild aortic valve accumulation ( $P < 0.05$ ).

OxPL levels. Unpaired Student  $t$  tests were also used to assess the differences in mean TBR, OxPL-apoB, and OxPL-apo(a) in patients with versus without elevated Lp(a) levels (on log-transformed values). TBR data of participants who deviated from the median by more than 3 standard deviation units were not included in the final analyses. Analyses of variance were used to test the differences in log-transformed Lp(a), OxPL-apoB, and OxPL-apo(a) according to estimated aortic valve calcium measured by echocardiography (mild, moderate, or severe). All statistical analyses were performed with SAS version 9.3 (SAS Institute Inc, Cary, NC), and a  $P$  value  $< 0.05$  was considered statistically significant.

## Results

Clinical characteristics of patients with and without CAVS are presented in Table 1. Patients with CAVS were on average slightly older than controls and had a more deteriorated cardiometabolic risk profile. Lp(a), OxPL-apoB, and OxPL-apo(a) levels in patients with versus without CAVS of the Quebec City cohort are presented in Figure 1. Figure 1 also presents the distribution of these biomarkers in patients with versus without CAVS. Compared with individuals without CAVS, patients with CAVS had higher Lp(a) (53.4%), OxPL-apoB (51.1%), and OxPL-apo(a) (59.6%).

**Table 3. Mean Lp(a) levels in patients with CAVS at various Lp(a) thresholds**

Lp(a) level categories (nmol/L)	Quebec cohort						Montreal cohort					
	Roche assay			UCSD assay			Roche assay			UCSD assay		
	N	%	Mean	N	%	Mean	N	%	Mean	N	%	Mean
≥ 325	1	0.6	415.2	2	1.1	346.5 ± 0.0	0	0	0	0	0	0
300-325	1	0.6	368.4 ± 66.3	1	0.6	334.4 ± 21.0	0	0	0	0	0	0
275-300	2	1.1	323.7 ± 64.2	2	1.1	315.3 ± 30.0	1	0.5	281.3	1	0.5	281.3
250-275	1	0.6	312.5 ± 61	3	1.7	294.9 ± 36.5	0	0	281.3	1	0.5	281.3
225-250	5	2.9	272.1 ± 58.9	1	0.6	288.7 ± 38.8	1	0.5	264.1 ± 24.4	2	0.9	264.1 ± 24.4
200-225	3	1.7	258.3 ± 57.4	5	2.9	261.7 ± 48.5	1	0.5	250.5 ± 29.2	3	1.4	250.5 ± 29.2
175-200	3	1.7	244.9 ± 59	12	6.9	228.6 ± 50.7	9	4.2	200.2 ± 33.2	12	5.6	200.2 ± 33.2
150-175	3	1.7	232.6 ± 61.3	6	3.4	216.3 ± 52.5	12	5.6	181.5 ± 30.4	24	11.2	181.5 ± 30.4
125-150	9	5.2	201.7 ± 68	10	5.7	197.1 ± 57.5	8	3.7	170.8 ± 32.4	32	14.9	170.8 ± 32.4
100-125	4	2.3	190.5 ± 70.2	10	5.7	180.4 ± 62.1	13	6.0	154.3 ± 38.0	45	20.9	154.3 ± 38.0
75-100	13	7.5	159.3 ± 77.1	4	2.3	173.9 ± 64.2	5	2.3	147.3 ± 41.8	50	23.3	147.3 ± 41.8
50-75	12	6.9	138.5 ± 79.5	10	5.7	156.6 ± 72.1	11	5.1	132.9 ± 49.0	61	28.4	132.9 ± 49.0
25-50	17	9.8	114.9 ± 82.2	26	14.9	121.5 ± 83	32	14.9	99.2 ± 61.4	93	43.3	99.2 ± 61.4
0-25	100	57.5	54.3 ± 74.9	82	47.1	68.5 ± 82.5	122	56.7	47.9 ± 60.5	215	100.0	47.9 ± 60.5
Total	174	100.0		174	100.0		215	100.0		215	100.0	

Lp(a), lipoprotein(a); UCSD, University of California, San Diego.

Mean Lp(a) (47.5 [interquartile range {IQR}, 6.3-70.3] nmol/L), OxPL-apoB (3.1 [IQR, 1.0-4.0] nmol/L), and OxPL-apo(a) (9.1 [IQR, 1.2-15.3] nmol/L) in patients from Montreal were also higher than in controls (all  $P < 0.05$ ).

In patients with CAVS, we found no evidence that Lp(a) was associated with CAVS severity. Table 2 presents the association between Lp(a), OxPL-apoB, and OxPL-apo(a) and CAVS severity criteria (peak aortic jet velocity, peak gradient, mean gradient, and indexed aortic valve area) measured by Doppler echocardiography. We observed higher Lp(a), OxPL-apoB, and OxPL-apo(a) levels in patients with higher amounts of aortic valve calcium, as estimated on echocardiography (Fig. 2).

The distribution of Lp(a) levels in various Lp(a) thresholds in patients with CAVS is presented in Table 3. Results presented in Table 3 suggest that in our cohorts, depending on the assay used to measure Lp(a) levels, 23.3% to 32.2% of patients with CAVS have Lp(a) levels  $\geq 75$  nmol/L, 18.4% to 29.9% have Lp(a) levels  $\geq 100$  nmol/L, 14.9% to 24.1% have Lp(a) levels  $\geq 125$  nmol/L, and 10.9% to 18.4% have Lp(a) levels  $\geq 150$  nmol/L. The mean values corresponding to these thresholds are presented in Table 3.

To further study the association between Lp(a) and AVC, we measured aortic valve microcalcification by 18F-NaF PET/CT in another cohort of 55 participants without established CAVS who had higher ( $\geq 75$  nmol/L,  $n = 27$ ) or lower ( $< 75$  nmol/L,  $n = 28$ ) Lp(a) levels. The clinical characteristics of these participants classified on the basis of Lp(a) levels are presented in Table 4. These characteristics of the study participants were similar with respect to age, sex, aortic valve function, and morphology. Participants with lower Lp(a) levels were more likely to be current or past smokers and had on average higher triglyceride levels compared with those with higher Lp(a) levels.

Figure 3A presents representative images of aortic valve microcalcification on 18F-NaF PET, comparing participants without established CAVS who had a lower or higher Lp(a) level. The aortic valves of both study participants do not present any sign of macrocalcification assessed by CT. However, the 18F-NaF PET/CT revealed strong evidence of microcalcification in the participant with a higher Lp(a) level, but not in the participant with a lower Lp(a) level. Quantification of the median aortic valve aortic score and 18F-NaF uptake (TBR) in the 2 groups described in Table 4 is presented in Fig. 3B and C, respectively. The mean TBR was 40% higher in individuals with higher (mean TBR =  $1.25 \pm 0.23$ ) versus lower (mean TBR =  $1.15 \pm 0.11$ ) Lp(a) levels ( $P = 0.02$ ). Similar differences were obtained when we investigated a higher threshold for Lp(a) levels (higher vs lower than 125 nmol/L; results not shown) and if we excluded participants with evidence of AVC ( $n = 25$ ). No differences in AVC accumulation were noted between individuals with high versus low Lp(a) levels.

**Discussion**

The results of our study suggest that patients with mild, moderate, or severe CAVS recruited in 2 contemporary “real-world” clinical settings, who did not undergo AVR, are characterized by higher levels of Lp(a), OxPL-apoB, and OxPL-apo(a) compared with individuals without CAVS. On

**Table 4. Clinical characteristics of individuals without CAVS with versus without elevated Lp(a) levels**

	Lp(a) < 75 nmol/L (n = 28)	Lp(a) ≥ 75 nmol/L (n = 27)	P value
<b>Clinical characteristics</b>			
Age, y	61.7 ± 6.4	59.8 ± 8.3	0.4
Men, n (%)	19 (68)	16 (59)	0.5
Body mass index, kg/m <sup>2</sup>	28.5 ± 4.3	28.8 ± 4.8	0.8
Waist circumference, cm	98.9 ± 12.3	99.1 ± 10.6	0.9
Systolic blood pressure, mm Hg	133.0 ± 14.8	126.1 ± 14.0	0.09
Diastolic blood pressure, mm Hg	83.3 ± 9.0	78.4 ± 10.6	0.07
Heart rate, beats/min	67.1 ± 10.4	69.0 ± 11.4	0.5
Diabetes, n (%)	2 (7)	4 (15)	0.4
CAD, n (%)	2 (7)	5 (19)	0.2
Hypertension, n (%)	9 (32)	13 (48)	0.2
Smoking (past or current), n (%)	19 (68)	10 (37)	0.02
Hyperlipidemia, n (%)	12 (43)	17 (63)	0.1
<b>Doppler echocardiographic data</b>			
Mean gradient, mm Hg	4.4 ± 1.6	4.6 ± 1.9	0.6
Peak gradient, mm Hg	8.4 ± 3.1	8.8 ± 3.3	0.6
Peak aortic jet velocity, cm/s	139.7 ± 25.6	144.8 ± 27.5	0.5
Indexed aortic valve area, cm <sup>2</sup> /m <sup>2</sup>	1.3 ± 0.3	1.7 ± 0.3	0.2
Aortic sclerosis, n (%)	4 (14)	6 (22)	0.4
<b>Laboratory data</b>			
Total cholesterol, mmol/L	5.0 ± 1.0	4.8 ± 1.2	0.6
Triglycerides, mmol/L	2.1 ± 1.1	1.6 ± 0.7	0.05
HDL-C, mmol/L	1.3 ± 0.5	1.4 ± 0.5	0.6
LDL-C, mmol/L	2.7 ± 0.9	2.7 ± 1.0	1.0
<b>CT data</b>			
Aortic valve calcium, n (%)	14 (50)	11 (40)	0.5
Median aortic valve calcium, AU	0.5 (0-35)	0 (0-11)	0.8

AU, Agatston units; CAD, coronary artery disease; CT, computed tomography; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Lp(a), lipoprotein(a).

Data are presented as mean ± standard deviation, median (interquartile range) or n (%).

investigation of the differences in plasma levels of Lp(a) and OxPL in these patients, we found that the distribution of these biomarkers was strikingly different in patients with versus without CAVS, because we noticed an important shift in Lp(a), OxPL-apoB, and OxPL-apo(a) toward higher values. To further characterize the association between Lp(a) and OxPL with valvular calcification, we assessed aortic valve microcalcification by 18F-NaF PET/CT and found that on average, despite having comparable aortic valve morphologies and little to no evidence of macrocalcification on CT, individuals with high Lp(a) level had a 40% higher uptake of 18F-NaF in the aortic valve compared with individuals with low Lp(a) levels.

The association between Lp(a) levels and the presence of aortic valve sclerosis and stenosis was first demonstrated in a cross-sectional analysis of the Cardiovascular Health Study, which included 5201 participants aged > 65 years.<sup>23</sup> The association between high Lp(a) levels and AVC, first reported by Thanassoulis et al.<sup>2</sup> in 2013, has been observed in various clinical settings, such as in patients with familial hypercholesterolemia.<sup>11</sup> In a case-control study of patients with versus without CAVS, Kamstrup et al.<sup>10</sup> documented a strong association between Lp(a), OxPL-apoB, and OxPL-apo(a) levels and CAVS risk in primary prevention settings. Similar associations with Lp(a), OxPL-apoB, and end-stage CAVS (patients undergoing AVR) were also reported by Nsaibia et al.<sup>7</sup> in a

cohort of patients with coronary artery disease. Moreover, our demonstration that patients with higher Lp(a) levels have evidence of developing aortic valve microcalcification even before any structural changes are seen on CT or echocardiography supports the concept that Lp(a) and OxPL might not only be associated with macroscopic AVC, CAVS, or AVR risk, but also could be implicated in the earliest steps of CAVS, even before the onset of macroscopic AVC.

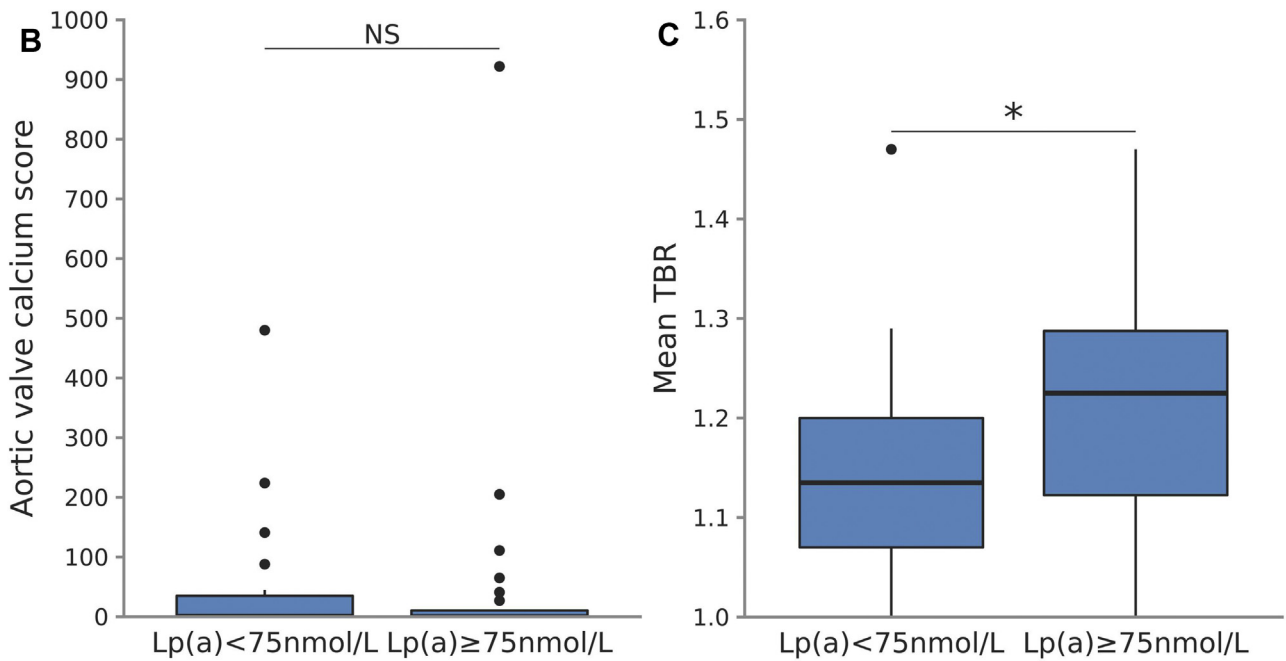
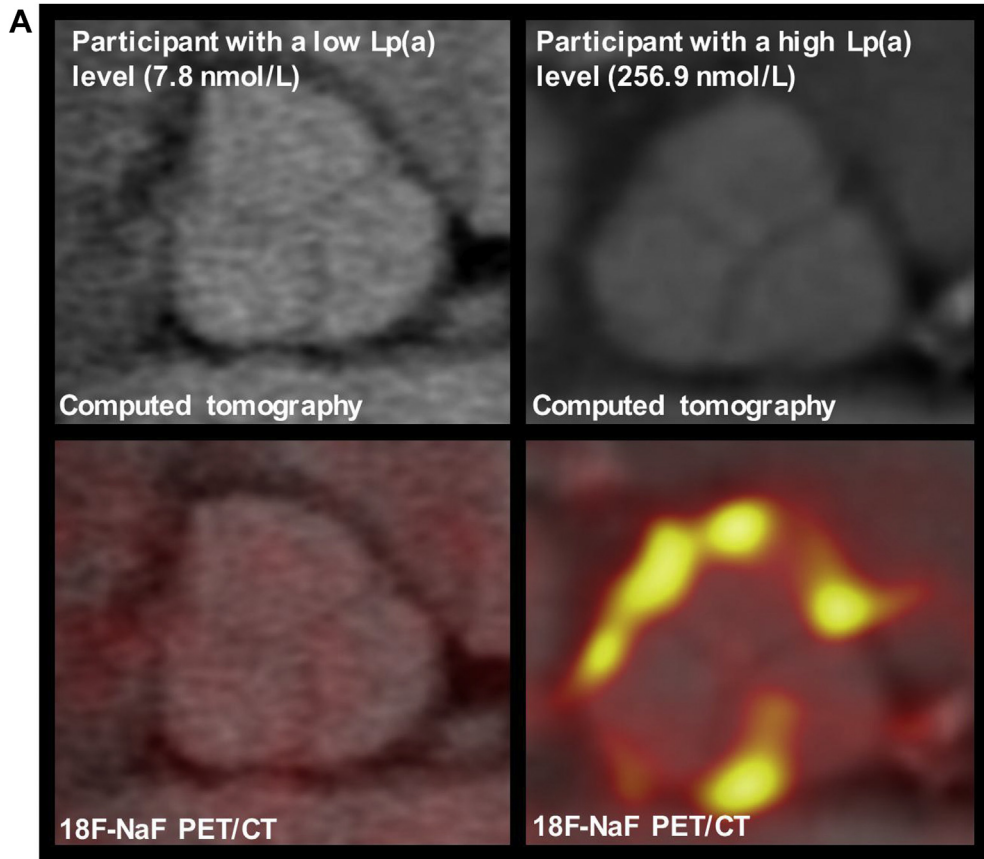
There are currently no other treatment options for CAVS aside from surgical removal of the aortic valve (AVR). Whether lowering Lp(a) or OxPL levels in patients with aortic sclerosis or mild to moderate CAVS will delay the progression of aortic valve microcalcification, AVC, or CAVS is unknown and should be investigated to confirm the causal role of Lp(a) in CAVS progression and to improve outcomes in patients with high Lp(a) levels and CAVS. Antisense oligonucleotides targeting *LPA* mRNA have been engineered and are currently being tested in patients with elevated Lp(a) levels. If Lp(a)-lowering trials were conducted in patients with various CAVS severity, based on the results of our study, approximately 23.3% to 32.2% of patients with CAVS could be enrolled on the basis of a sufficiently elevated Lp(a) level if the Lp(a) threshold was set at 75 nmol/L (assuming that patients meet all other entry criteria). If more conservative thresholds were selected, one could expect to enroll 18.4% to 29.9% of patients with CAVS with Lp(a) levels ≥ 100 nmol/L, 14.9% to 24.1% of patients with CAVS with Lp(a) levels ≥ 125 nmol/L, or 10.9% to 18.4% of patients with CAVS with Lp(a) levels ≥ 150 nmol/L. Our results suggest that different cohorts and different Lp(a) assays will likely contribute to the variation in the number of participants needed to screen for such trials.

By showing that individuals without CAVS with high Lp(a) levels may be characterized by higher aortic valve microcalcification assessed by 18F-NaF PET/CT, our results also suggest for the first time that Lp(a)/OxPL might be implicated in CAVS disease initiation, before the onset of macroscopic calcification of the aortic valve, a novel finding. 18F-NaF PET/CT is a noninvasive tool to assess active aortic valve pathological mineralization triggered by inflammation and cell death. 18F-NaF binds to active calcified nodules through chemical reactions with hydroxyapatite, a crystalline structure of calcium and phosphates.<sup>24</sup> Additionally, 18F-NaF is an economical PET ligand that is relatively easy to manufacture and is detectable below the detection limit of CT. This imaging outcome is used in the SALTIRE II trial, which will document the impact of drugs targeting osteoporosis in the progression of CAVS (NCT02132026).

### Study strengths and limitations

Although the number of individuals included in our study might be suboptimal to represent the entire CAVS population, strengths of our study include the inclusion of a “real-world” CAVS population selected in a contemporary clinical setting. Therefore, we believe that our results might underestimate CAVS prevalence observed in the settings of patients with very high Lp(a) levels, for instance, above the 95th percentile of the population distribution of Lp(a). The overwhelming majority of our study population was white. The prevalence of patients with Lp(a) levels above certain thresholds might have been higher had we included individuals of





**Figure 3.** (A) Representative image of aortic valve microcalcification in a patient with a low Lp(a) level (computed tomography [CT] at the **top left** and positron emission tomography [PET]/CT at the **bottom left**) and a patient with a high Lp(a) level (CT at the **top right** and PET/CT at the **bottom right**). (B) Mean aortic valve calcium score in individuals with high versus low Lp(a) levels. (C) Mean 18F-sodium fluoride (NaF) uptake (tissue-to-background ratio [TBR]) in individuals with high versus low Lp(a) levels. Lp(a), lipoprotein(a). \**P* = 0.02.

black or South Asian ancestries. Other limitations of our study include the cross-sectional rather than a prospective study design and the estimation of AVC by echocardiography instead of CT in patients with CAVS. Additionally, although aortic valve NaF uptake has been shown to correlate with later-stage AVC accumulation and histological markers of CAVS severity in a small study,<sup>25</sup> it would be important for other studies to determine whether microcalcification in the setting of high Lp(a) levels would eventually result in faster disease progression and the development of overt CAVS in an adequately powered study.

## Conclusion

The results of our study suggest that elevated Lp(a) and OxPL levels are associated with prevalent CAVS in patients studied in a “real-world” echocardiography laboratory setting. In patients with elevated Lp(a), evidence of aortic valve microcalcification by 18F-NaF PET/CT is present before the development of macroscopic AVC and clinically manifested CAVS, suggesting the possibility to detect early events in disease initiation.

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