ORIGINAL RESEARCH

ApoCIII-Lp(a) complexes in conjunction with Lp(a)-OxPL predict rapid progression of aortic stenosis

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ABSTRACT

Objective This study assessed whether apolipoprotein CIII-lipoprotein(a) complexes (ApoCIII-Lp(a)) associate with progression of calcific aortic valve stenosis (AS).

Methods Immunostaining for ApoC-III was performed in explanted aortic valve leaflets in 68 patients with leaflet pathological grades of 1–4. Assays measuring circulating levels of ApoCIII-Lp(a) complexes were measured in 218 patients with mild–moderate AS from the AS Progression Observation: Measuring Effects of Rosuvastatin (ASTRONOMER) trial. The progression rate of AS, measured as annualised changes in peak aortic jet velocity (V_{peak}), and combined rates of aortic valve replacement (AVR) and cardiac death were determined. For further confirmation of the assay data, a proteomic analysis of purified Lp(a) was performed to confirm the presence of apoC-III on Lp(a).

Results Immunohistochemically detected ApoC-III was prominent in all grades of leaflet lesion severity. Significant interactions were present between ApoCIII-Lp(a) and Lp(a), oxidised phospholipids on apolipoprotein B-100 (OxPL-apoB) or on apolipoprotein (a) (OxPL-apo(a)) with annualised V_{peak} (all $p<0.05$). After multivariable adjustment, patients in the top tertile of both apoCIII-Lp(a) and Lp(a) had significantly higher annualised V_{peak} ($p<0.001$) and risk of AVR/cardiac death ($p=0.03$). Similar results were noted with OxPL-apoB and OxPL-apo(a). There was no association between autotaxin (ATX) on ApoB and ATX on Lp(a) with faster progression of AS. Proteomic analysis of purified Lp(a) showed that apoC-III was prominently present on Lp(a).

Conclusion ApoC-III is present on Lp(a) and in aortic valve leaflets. Elevated levels of ApoCIII-Lp(a) complexes in conjunction with Lp(a), OxPL-apoB or OxPL-apo(a) identify patients with pre-existing mild–moderate AS who display rapid progression of AS and higher rates of AVR/cardiac death.

Trial registration NCT00800800.

INTRODUCTION

Calcific aortic valve stenosis (AS) is associated with significant morbidity and mortality.[1] Due to lack of therapies that slow progression of pre-existing AS, conventional surgical or transcatheter aortic valve replacement (AVR) is the only therapy currently available. However, in many patients, AVR is used late in life when comorbidities are common, and prognosis can be poor despite surgery.[2]

The prevalence of AS is growing, with an estimated 4.5 million cases of clinically significant AS estimated by 2030.[3] Certain risk factors for AS have been recognised for many years, including age, hypertension, metabolic syndrome and elevated low-density lipoprotein cholesterol (LDL-C).[4] However, four randomised trials of statin therapy in patients with modest elevations of LDL-C have not shown any difference in AS progression rates or the need for AVR, suggesting that other aetiologic factors are more causally involved.[5] Genetic studies have shown that single nucleotide polymorphisms in the *LPA* gene are associated with elevated lipoprotein(a) [Lp(a)] levels and aortic valve calcification and AS.[6–8] It has also been shown that oxidised phospholipids (OxPL) associated with apolipoprotein B-100 (apoB) and Lp(a) are associated with AS, potentially as causal mediators with a linear relationship of OxPL-apoB levels with echocardiographically determined progression and need for AVR.[9–11] Furthermore, autotaxin (ATX) mass and activity, which generate pro-calcifying lysophosphatidic acid from breakdown products of OxPL are also associated with AS and evidence suggest that ATX is transported by Lp(a).[10 12 13] Metabolic syndrome, which includes elevated triglycerides, is also associated with AS.[14] Elevated levels of apolipoprotein C-III (ApoC-III) are associated with elevated triglyceride levels and remnant cholesterol[15] and are also associated with higher risk of cardiovascular disease.[16–18]

Clinical and biochemical measures that predict the progression of established AS are suboptimal. We have developed novel assays to integrate various lipoproteins in the pathophysiological relationships in AS and have applied them to the AS Progression Observation: Measuring Effects of Rosuvastatin (ASTRONOMER) trial. Specifically, we have developed assays to measure complexes of ApoC-III on Lp(a) [ApoCIII-Lp(a)][19] and assessed their role in progression of AS and need for AVR. Additionally, we have developed high-throughput ELISAs to measure ATX mass on Lp(a) [ATX-Lp(a)] and apolipoprotein B-100 [ATX-apoB].[10]

METHODS

Patient population

The design and main results of the ASTRONOMER trial have been previously reported[20] and detailed

description of the inclusion and exclusion criteria is presented in the online supplementary material. The study protocol was approved by the institutional review boards at all participating centres and patients gave written signed informed consent. Of the 269 patients enrolled in ASTRONOMER, 218 had remaining blood samples for the current analysis.

Study outcomes

The primary outcome for this study was the progression rate of AS measured as annualised changes in peak aortic jet velocity (V_{peak}). To account for different follow-up lengths, annualised V_{peak} was calculated by dividing the difference between last follow-up and baseline values by the length of follow-up. The secondary outcome was the composite of AVR or cardiac death. Finally, based on explanted valves from surgery, the presence of ApoC-III and its co-localisation of Lp(a) and OxPL were tested.

Clinical, laboratory and Doppler echocardiographic data

Clinical, laboratory and Doppler echocardiographic data for the ASTRONOMER trial are previously described (online supplementary material).[20]

Aortic valve morphology and function were assessed by Doppler echocardiography and the primary echocardiographic parameter to assess AS severity was peak aortic jet velocity (V_{peak}) (online supplementary material).[9 14]

Quantification of circulating biomarkers and immunostaining related to ApoC-III and ATX

Detailed methodology related to the antibodies used and the assays performed to quantify circulating biomarkers are presented in the online supplementary material.

Assay methodology in cartoon format for ApoCIII-Lp(a), -ATX-Lp(a) and ATX-ApoB is shown in online supplementary figure 1 and results are reported as relative light units (RLU) in 100 ms.

Proteomic analysis of purified Lp(a)

Lp(a) was purified from the lipid apheresis eluate of three distinct subjects undergoing LDL apheresis. Detailed methodology related to the Lp(a) proteomic analysis and LC-MS/MS method used is presented in the online supplementary material.

Histological and immunohistochemical analysis of valve leaflets following surgical AVR

Detailed methodology of the analysis of the 68 patients recruited at the Robert-Bosch-Hospital (Stuttgart, Germany) is presented in the online supplementary material.

Statistical analysis

Continuous data were expressed as mean±SD and were tested for normality of distribution and homogeneity of variances with the Shapiro–Wilk and Levene tests, respectively. Plasma levels of total ApoC-III, ApoCIII-Lp(a), ATX-apoB and ATX-Lp(a), Lp(a), OxPL-apoB and OxPL-apo(a) were reported as median (IQR). Categorical variables were expressed as percentages. The Spearman's rank correlations were used to determine the association between total ApoCIII, ApoCIII-Lp(a), ATX-apoB and ATX-Lp(a) with (i) the standard lipid biomarkers (ie, LDL-C, corrected LDL-C for Lp(a) cholesterol content, ApoB, HDL-C and TG); (ii) the glycaemic biomarkers (ie, fasting glucose, insulin and HOMA-IR) and (iii) Lp(a), OxPL-apoB and OxPL-apo(a).

For the analysis of the primary outcome (annualised V_{peak}), progression rate of AS was compared across the top tertile and the two bottom tertiles for total ApoC-III (>10.6 mg/dL vs ≤10.6 mg/dL), ApoCIII-Lp(a) (>2 97 000 RLU vs ≤2 97 000 RLU), ATX-apoB (>5600 RLU vs ≤5600 RLU) and ATX-Lp(a) (>5900 RLU vs ≤5900 RLU) using unpaired Student's t-test or Mann–Whitney U test, as appropriate. Univariable and multivariable linear regression analyses were performed to assess the independent association between baseline total ApoC-III, ApoCIII-Lp(a), ATX-apoB and ATX-Lp(a) plasma levels (ie, defined as top tertile vs middle and bottom tertiles) and (i) baseline AS severity and (ii) AS progression defined as annualised V_{peak}. The multivariable models were built according to our previous analysis and included the following variables: (1) variables with p value<0.10 in individual analysis, (2) traditional cardiovascular risk factors, (3) aortic valve phenotype (bicuspid vs tricuspid) and (4) randomisation status (statin vs placebo).[9 14] Results were reported as standardised βeta coefficient ±SE (βeta±SE). Univariable and multivariable logistic regression models were performed to identify the risk of being *rapid progressor* defined as an annualised progression rate of V_{peak}≥0.20 m/s/yr as previously described.[9] Results were reported as ORs with 95% CIs.

Interactions with regard to progression rate of AS between total ApoC-III, ApoCIII-Lp(a), ATX-apoB and ATX-Lp(a) levels,

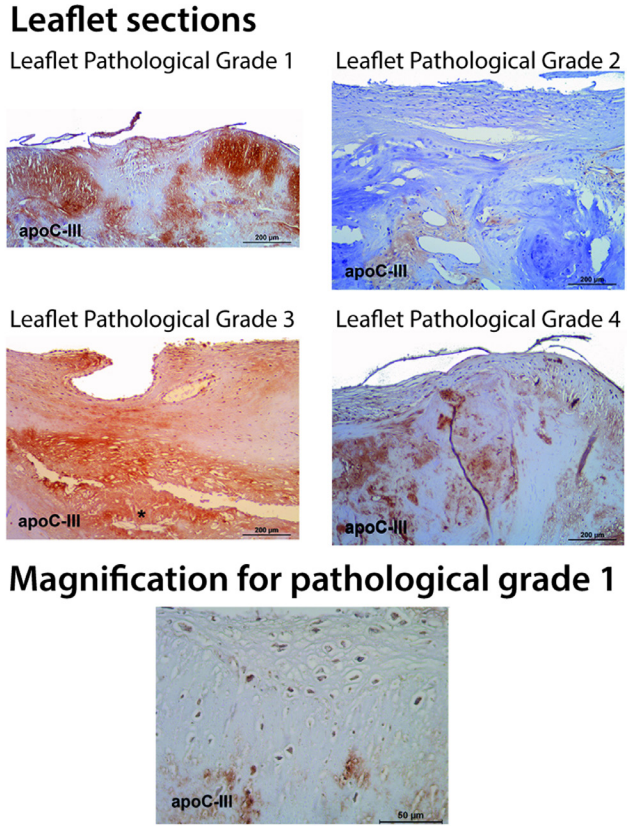

Figure 1 Representative immunohistological staining for ApoC-III according to pathological grading of explanted aortic valve leaflets. Part A shows aortic valve leaflet sections stained for apolipoprotein C-III (apoC-III). In all panels, the aortic side of the valve is to the top. Note the presence of ApoC-III in all pathological grades, and the increase in the staining with the increase in the pathological severity of the lesion. Part B shows higher magnification of representative immunohistological staining for ApoC-III according to pathological grade 1 explanted aortic valve leaflets. Individual selected areas from the top panel in part A stained for apolipoprotein C-III (apoC-III) at higher magnification with visibility of cell nuclei.

Table 1 Baseline characteristics of patients in the AS Progression Observation: Measuring Effects of Rosuvastatin trial

Variables	All patients n=218
Clinical	
Age, years	58±13
Male gender, %	60%
Height, cm	169±10
Weight, kg	81±17
Body surface area, m^2	1.91±0.21
Body mass index, kg/m^2	28±6
Waist circumference, cm	95±14
Metabolic syndrome, %	27%
History of hypertension, %	32%
Systolic blood pressure, mm Hg	127±16
Diastolic blood pressure, mm Hg	75±10
History of smoking, %	49%
Medication	
Anti-hypertensive treatment, %	18%
ACE inhibitors, %	11%
ARBs, %	7%
Rosuvastatin, %	51%
Laboratory data	
LDL-C, mg/dL	126±27
Corrected LDL-C, mg/dL*	116±27
ApoB, mg/dL	102±19
HDL-C, mg/dL	58±17
Triglycerides, mg/dL	120±61
Fasting glucose, mg/dL	95±11
Creatinine, mg/dL	0.91±0.19
Lp(a), mg/dL	29.9 [12.6–76.3)
OxPL-apoB, nM	3.54 [2.27–8.67)
OxPL-apo(a), nM	15.9 [4.46–53.4)
ATX-apoB, RLU	4669 [3625–5910)
ATX-Lp(a), RLU	3679 [2268–8338)
Total ApoCIII, mg/dL	8.79 [6.33–11.47)
ApoCIII-Lp(a), RLU	2 30 653 [1 75 031–3 34 8)
Doppler echocardiographic data	
Bicuspid aortic valve, %	48%
Aortic valve calcification score	1.7±0.7
Peak aortic jet velocity, m/s	3.2±0.4
Peak transvalvular gradient, mm Hg	41±11
Mean transvalvular gradient, mm Hg	22±7
Aortic valve area, cm^2	1.34±0.42
Indexed aortic valve area, cm^2/m^2	0.70±0.21
Valvulo-arterial impedance, mm Hg/mL.$m^{2.04}$	4.9±1.4
Relative wall thickness ratio	0.44±0.09
LV mass index, $g/m^{2.7}$	48±15
LV ejection fraction, %	66±7

Values are mean ±SD or median (IQR).
*LDL-C was corrected for the cholesterol content in Lp(a) using the following formula: corrected LDL-C=LDL C – Lp(a) mass in mg/dL×0.3.
ACE, angiotensine-converting enzyme; apo(a), apolipoprotein (a); apoB, apolipoprotein B; apoCIII, apolipoprotein CIII; ARBs, angiotensin receptor blockers; ATX, autotaxin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Lp(a), lipoprotein(a); LV, left ventricle; OxPL, oxidised phospholipids; RLU, relative light units.

with biomarkers we had previously identified as predictors of rapid progression of AS (ie, Lp(a), OxPL-apoB and OxPL-apo(a) levels),[9] were determined using linear regression models. Two-way analysis of variance followed by Tukey's post-hoc test was then performed to compare annualised V_{peak} across groups. The independent predictive value of the combined 'top tertile of ApoCIII-Lp(a)-top tertile of Lp(a)' and 'top tertile of ApoCIII-Lp(a)-top tertile of OxPL-apoB' groups was assessed using multivariable linear and logistic regression analyses, as described above.

For the analysis of the secondary outcome (composite of AVR or cardiac death; n=48), univariable and multivariable Cox proportional hazard models were performed to assess the association between 'top tertile of ApoCIII-Lp(a)-top tertile of Lp(a)' group and AS-related events. The proportional hazards assumption was checked with the use of Schoenfeld residuals. Models were adjusted for age, gender and baseline AS severity, and results were reported as HRs with 95% CI. A p value<0.05 was considered statistically significant.

RESULTS

Histological and immunohistochemical findings from surgically explanted aortic valve leaflets

As previously reported, the 68 aortic valves were pathologically classified as grades 1–4.[10] ApoC-III was detectable in every lesion examined, from grades 1 to 4, at low (figure 1A) and high (figure 1B) magnification. A more abundant deposition of apoC-III was observed with the increase in pathological grades, especially in grades 3–4 (figure 1A). ApoC-III co-localised with apo(a) staining through all degree of lesions severity, as well as with OxPL epitopes in the more severe pathological valve (online supplementary figure 2). Negative control stains with both normal rabbit serum and irrelevant isotype-matched antibodies were negative (online supplementary figure 3).

Population characteristics of the ASTRONOMER trial and lipids biomarkers

Baseline clinical, laboratory and Doppler echocardiographic characteristics of the entire population in ASTRONOMER are presented in table 1.

There were modest but significant correlations between plasma levels of ApoCIII-Lp(a) and all the standard lipid biomarkers (ie, LDL, LDL-corr, apoB, HDL, TG), as well as with insulin and HOMA-IR, the strongest being between ApoCIII-Lp(a) and TG (r_s=0.48, p≤0.005; online supplementary table 1). The plasma level of total ApoC-III was also significantly correlated with lipid biomarkers, except for HDL-C. ATX-apoB presented a negative correlation with Lp(a) (r_s=−0.15; p=0.02). Negative correlations were also observed between ATX-Lp(a) and glycaemic biomarkers (ie, fasting glucose, insulin and HOMA-IR) but all the other correlations tested were not significant. Total ApoC-III and ApoCIII-Lp(a) were also significantly correlated with ATX-apoB (r_s=0.33 and r_s=0.34, respectively, p<0.001; online supplementary table 1).

Table 2 P values of the interaction between ApoCIII and autotaxin biomarkers vs Lp(a), OxPL-apoB, OxPL-apo(a) for annualised progression rate of as (V_{peak})

	Total ApoC-III	ApoCIII-Lp(a)	ATX-ApoB	ATX-Lp(a)
Lp(a)	0.19	**0.01**	0.06	0.68
OxPL-apoB	0.13	**0.01**	**0.03**	0.97
OxPL-apo(a)	0.24	**0.02**	**0.05**	0.93

apo(a), apolipoprotein (a); apoB, apolipoprotein B; apoCIII, apolipoprotein CIII; AS, aortic stenosis; ATX, autotaxin; Lp(a), lipoprotein(a); OxPL, oxidised phospholipids; V_{peak}, peak aortic jet velocity.

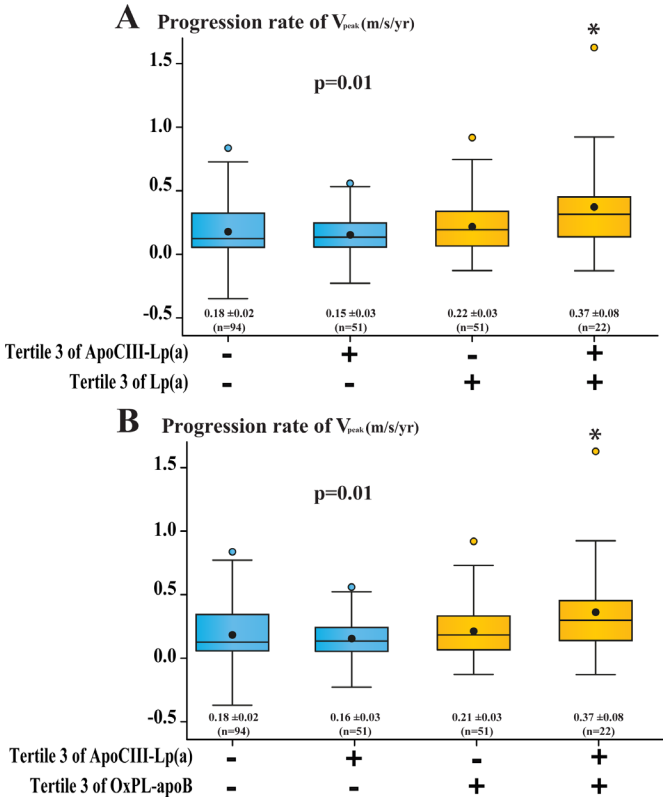

Figure 2 AS progression rate according to top tertiles of ApoCIII-Lp(a) and Lp(a) or OxPL-apoB. Comparison of annualised Vpeak after dichotomisation by middle and bottom tertiles versus top tertile of ApoCIII-Lp(a) (ie, apoCIII-Lp(a)≤ vs >297,000 RLU) and tertile of Lp(a) (ie, interaction between tertiles of ApoCIII-Lp(a) and Lp(a); (A) or tertile of OxPL-apoB (ie, interaction between tertiles of ApoCIII-Lp(a) and OxPL-apoB; (B). ApoCIII, apolipoprotein C-III; Lp(a), lipoprotein(a); Vpeak, peak aortic jet velocity. P values are from two-way ANOVA. *p<0.05 compared with the other three groups (from Tukey's post hoc test). The box shows 25th and 75th percentiles, the median line shows the median value and the black dot the mean value, error bars the 10th and 90th percentiles; circles are outliers. The numbers at the bottom of the graph are mean±SEM of the progression rate of Vpeak and between brackets the number of patients in each group. ApoCIII, apolipoprotein CIII; apoB, apolipoprotein B; ANOVA, analysis of variance; AS, aortic valve stenosis; Lp(a), Lipoprotein(a); OxPL, oxidised phospholipids; RLU, relative light units.

There was no significant association between total ApoC-III, ApoCIII-Lp(a), ATX-apoB and ATX-Lp(a) and baseline V_{peak}, mean gradient (MG) or aortic valve area (AVA) (all p>0.20). There was no effect of statin therapy during the first-year post randomisation on ApoC-III or ATX biomarkers (all p>0.16).

AS progression rate and total ApoC-III, ApoCIII-Lp(a), ATX-ApoB and ATX-Lp(a) biomarkers

The median echocardiographic follow-up was 3.5 (2.9–4.5) years. There was no difference in annualised V_{peak} between top tertile versus middle and bottom tertiles of total ApoC-III (0.23±0.26 versus 0.19±0.22, p=0.27; online supplementary figure 4A) and of ApoCIII-Lp(a) (0.22±0.28 vs 0.19±0.21, p=0.47, online supplementary figure 4B), as well as top tertile versus middle and bottom tertile of ATX-apoB or ATX-Lp(a) (all p>0.80). After comprehensive multivariable adjustment, results were consistent (all p>0.20; online supplementary table 2). Similar results were observed with the analysis looking at the risk of being *rapid* progressors (all p>0.15; online supplementary table 2). There was no impact of statin treatment for all biomarkers (all p>=0.29).

Interaction between total ApoC-III, ApoCIII-Lp(a), ATX-apoB and ATX-Lp(a) biomarkers and Lp(a), OxPL-apoB or OxPL-apo(a) with AS progression rate

There were significant interactions between ApoCIII-Lp(a) and Lp(a) (p=0.01), OxPL-apoB (p=0.01) or OxPL-apo(a) (p=0.02) with regards to AS progression rate (table 2). Interactions were borderline significant for ATX-apoB (p≤0.06), and not significant with total ApoC-III and ATX-Lp(a) (p≥0.13; table 2).

The annualised V_{peak} was twofold faster in the combined group of top tertile of ApoCIII-Lp(a) and top tertile of Lp(a) (ie, 'tertile 3 of ApoCIII-Lp(a)-tertile 3 of Lp(a)' group; n=22) as opposed to the other groups (figure 2A). After comprehensive multivariable adjustment, 'tertile 3 of ApoCIII-Lp(a)-tertile 3 of Lp(a)' was the only group which remained significantly associated with annualised V_{peak} (βeta coeff.: 0.28±0.07, p=0.001). The interaction between ApoCIII-Lp(a) and OxPL-apoB (figure 2B) or OxPL-apo(a) showed similar results. In multivariable analysis, the combined group of top tertile of ApoCIII-Lp(a) and top tertile of OxPL-apoB (βeta coeff.: 0.29±0.07, p=0.001) was associated with higher annualised V_{peak}. Similar results were observed for OxPL-apo(a) (p≤0.02; data not shown).

There was no significant interaction between ApoC-III or ATX biomarkers and age, bicuspid aortic valve phenotype or the presence of metabolic syndrome with regard to AS progression rate (all p>0.05).

All these results were confirmed by the analysis of the risk of being a rapid *progressor*, defined as an annualised V_{peak}≥0.20 m/s/yr (detailed numbers of *rapid progressor* in each studied

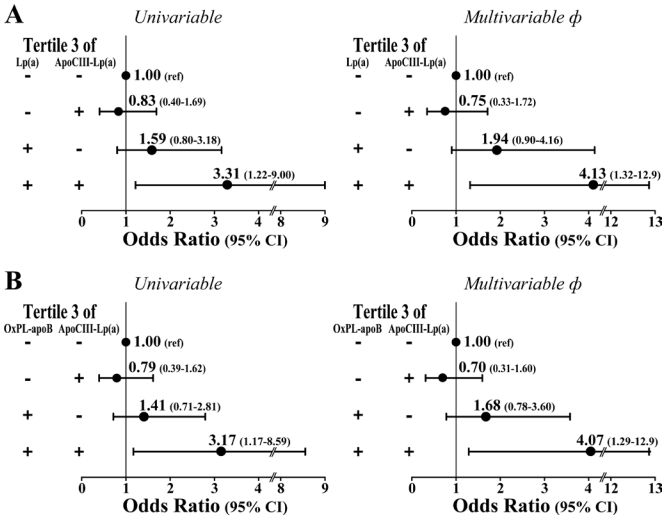

Figure 3 Risk of rapid AS progression according to top tertiles of ApoCIII-Lp(a) and Lp(a) or OxPL-apoB. Univariable and multivariable ORs for the risk of being a rapid progressor (ie, annualised V_{peak}>0.20 m/s/yr) in the four groups of patients defined according to middle and bottom tertiles versus top tertile of ApoCIII-Lp(a) and Lp(a) (A) or OxPL-apoB (B). ϕ Multivariable adjustment for age, gender, hypertension, metabolic syndrome, statin use, corrected LDL-C, creatinine, bicuspid aortic valve phenotype, aortic valve calcification score and baseline peak aortic jet velocity. ApoCIII, apolipoprotein CIII; apoB, apolipoprotein B; AS, aortic valve stenosis; Lp(a), Lipoprotein(a); OxPL, oxidised phospholipids.

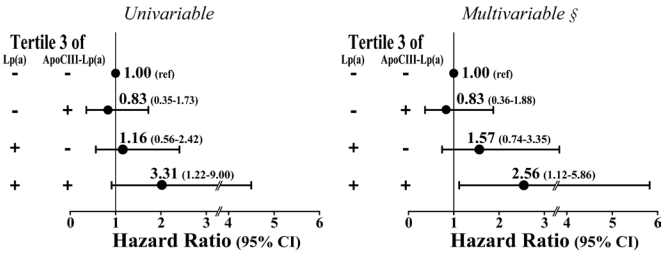

Figure 4 Risk of aortic valve-related events according to top tertiles of ApoCIII-Lp(a) and Lp(a). Univariable and multivariable Hazard Ratios for the composite of AVR or cardiac death in the four groups of patients defined according to middle and bottom tertiles versus top tertile of ApoCIII-Lp(a) and Lp(a). § Multivariable adjustment for age, gender and baseline peak aortic jet velocity. ApoCIII, apolipoprotein CIII; AVR, aortic valve replacement; Lp(a), lipoprotein(a).

group are presented in the online supplementary table 3). The 'tertile 3 of ApoCIII-Lp(a)-tertile 3 of Lp(a)' was the only group associated with a higher risk of rapid AS progression in univariable and multivariable analyses (OR=3.3, 95% CI: 1.2 to 9.0, p=0.02; and OR=4.1, 95% CI: 1.3 to 12.9, p=0.02; respectively) (figure 3A). Similarly, 'tertile 3 of ApoCIII-Lp(a)-tertile 3 of OxPL-apoB' provided consistent results in univariable and multivariable analyses (OR=3.2, 95% CI: 1.2 to 8.6, p=0.02; and OR=4.1, 95% CI: 1.3 to 12.9, p=0.02; respectively; figure 3B). Similar results were obtained for OxPL-apo(a) (data not shown).

Relationship of ApoCIII-Lp(a) with AS-related clinical events

During follow-up, 48 AS-related events occurred, including 46 AVR and two cardiac deaths (detailed numbers of events in each studied group are presented in online supplementary table 3). After adjustment for age, gender and baseline AS severity, 'tertile 3 of ApoCIII-Lp(a)-tertile 3 of Lp(a)' group was the only one associated with a higher risk of clinical events (HR=2.56, 95% CI 1.12 to 5.86, p=0.03; figure 4).

Lp(a) Proteome

LC-MS/MS analysis of isolated, purified Lp(a) coming from three distinct patients showed that apoC-III accounted for 3.62% of the total ion intensity of all apolipoproteins. When the total ion intensity was adjusted for the molecular weight of the protein, the proportion of apoC-III on Lp(a) was 20.44% (table 3), the highest of all lipoproteins, suggesting multiple copies of apoC-III are present on Lp(a). A variety of other apolipoproteins are also present, including apoE, apoC-I, apoAII and apoC-II. A list of the most readily detected proteins is shown in online supplementary table 4.

DISCUSSION

The current study demonstrates that ApoC-III is a prominent component of Lp(a) and has strong immunological presence in aortic valve leaflets and co-localises with Lp(a) and OxPL. Furthermore, the study expands our knowledge on the determinants of rapid progression of mild–moderate AS by showing that apoCIII-Lp(a) complexes in conjunction with either Lp(a) or OxPL-apoB and OxPL-apo(a) identify rapid progressors echocardiographically and patients at highest risk for AVR. This study advances our ability to predict the progression of pre-existing mild-moderate AS by combining lipid, metabolic and inflammatory components into a biomarker construct. These findings may prove its usefulness in interpreting future studies of AS.

Table 3 LC-MS/MS analysis of Lp(a) unambiguously identified ApoC-III

	Lp(a) apolipoprotein composition	
	Total ion intensity (%)	PSM/Mw (%)
LPA	22.87	2.48
APOAI	0.94	5.31
APOAII	0.80	7.04
APOAIV	0.03	1.87
APOB	60.10	16.07
APOCI	0.74	11.92
APOCII	0.85	5.74
APOCIII	**3.62**	**20.44**
APOCIV	0.08	3.04
APOD	0.34	4.89
APOE	6.21	15.22
APOL1	0.03	1.18
APOM	0.24	3.72
CLU	0.13	1.38

LC-MS/MS analysis of Lp(a) from three distinct patients unambiguously identified Apo-CIII. Spectra for Apo-CIII accounted for 3.62% of the total ion intensity of apolipoproteinsin the LC-MS/MS analysis of Lp(a). Given the small size of apoC-III, the peptide spectral matches presented in online supplementary table 3 were also adjusted for molecular weight of the protein.

It is known that ApoC-III circulates on all lipoproteins, including Lp(a).[19 21] However, unlike apoB on LDL or apo(a) on Lp(a) where there is a 1:1 relationship, multiple particles of ApoC-III may be carried by various lipoproteins.[22] Interestingly, in the current study and in a prior study where methods to measure ApoCIII-Lp(a) complexes were first described,[19] there was no correlation of ApoCIII-Lp(a) and Lp(a) levels. This suggests that only a subset of Lp(a) particles carry apoC-III, and if these patients concomitantly have elevated Lp(a), OxPL-apoB or OxPL-apo(a), they are at high risk of AS progression.

The patients in the ASTRONOMER trial had mild–moderate AS at enrolment, with baseline mean peak aortic jet velocity of 3.2 m/s and valve area 1.34 cm^2. In a prior analysis from ASTRONOMER, subjects in the highest tertile of Lp(a), OxPL-apoB or OxPL-apo(a), had AS progression rates of 0.26 m/s/yr.[9] In the current study, subjects in the highest tertile of apoCIII-Lp(a) and Lp(a) or OxPL-apoB had rates of progression of 0.37 m/s/yr, the highest progression rate seen with any variable previously studied in ASTRONOMER, including age, hypertension, elevated LDL-C, body mass index and metabolic syndrome (summary figure 5).[14]

Prior studies in ASTRONOMER showed that metabolic syndrome was associated with faster stenosis progression and poorer prognosis and that it was significant only in patients<57 years of age.[14] The current findings are also consistent with the relationship of Lp(a), OxPL-apoB and OxPL-apo(a), which are primarily genetic risk factors[23] and are also more prominent predictors in similarly younger patients.[9] It was previously shown that apoB and apoE are present in aortic valve leaflets,[24] and the full proteome of aortic valve leaflets is slowly being defined.[25] ApoC-III is carried by all apoB and apoE-containing lipoproteins, so it follows that these lipoproteins are all present in Lp(a) particles as well as in aortic valve leaflets.[21]

ATX-apoB and ATX-Lp(a) were not predictors of progression of AS. In prior studies, ATX activity was elevated by 60% in mineralised aortic valves in comparison with control valves.[12] Immunohistochemistry studies showed a high level of ATX in mineralised aortic valves, which co-localised with OxPL and

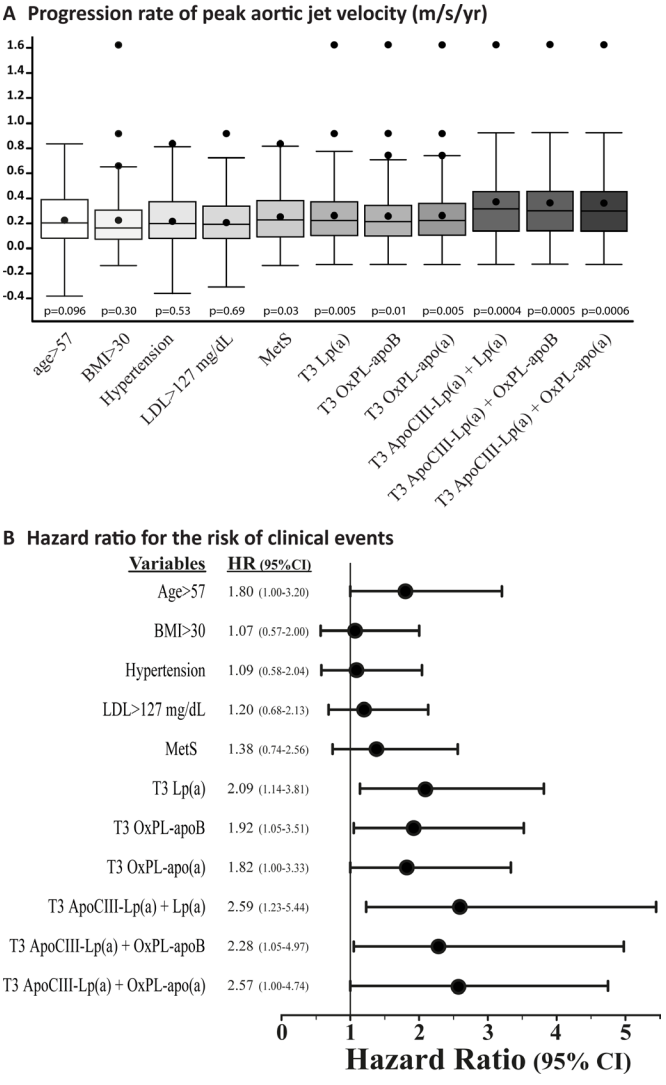

Figure 5 Comparison of annualised V_{peak} (m/s/yr) and Hazard Ratios for the risk of clinical events for various risk factors. *:* progression rates of V_{peak} (A) and HRs for risk of clinical events defined as AVR or cardiac death (B, where HRs are adjusted for age, sex and baseline as severity) in 218 patients from the ASTRONOMER trial. Data are presented for the subset of older patients (ie, age ≥median age; n=110), obese patients (ie, body mass index ≥30 kg/m²; n=64), patients with hypertension (n=70), higher LDL-C (ie, LDL-C >median LDL-C; 109), metabolic syndrome (n=57), top tertile of Lp(a), OxPL-apoB and OxPL-apo(a) (n=73 for all three groups), and the combined groups of 'top tertile of ApoCIII-Lp(a)-top tertile of Lp(a)', 'top tertile of ApoCIII-Lp(a)-top tertile of OxPL-apoB' and 'top tertile of ApoCIII-Lp(a)-top tertile of OxPL-apo(a)' (n=22 for all three groups). For the (A), the box shows 25th and 75th percentiles, the median line shows the median value and the black dot the mean value, error bars the 10th and 90th percentiles; circles are outliers. P values highlight the significance when comparing patients with the specific risk factor versus those without (ie, age <57 years, BMI <30, no hypertension, lower LDL-C, no MetS, middle and bottom tertiles of Lp(a), OxPL-apoB and OxPL-apo(a), and the other three groups of patients without both elevated ApoCIII-Lp(a) and Lp(a), OxPL-apoB or OxPL-apo(a)). Apo(a), apolipoprotein (a); ApoB, apolipoprotein B; ApoCIII, apolipoprotein CIII; ASTRONOMER, AS Progression Observation: Measuring Effects of Rosuvastatin; AVR, aortic valve replacement; BMI, body mass index; LDL-C, low-density lipoprotein-cholesterol; Lp(a), lipoprotein(a); MetS, metabolic syndrome; OxPL, oxidised phospholipids.

apo(a).[10] ATX activity was also present in the isolated Lp(a) fraction of patients without AS.[12] Furthermore, valve interstitial cells expressed ATX and lysophosphatidic acid promoted the mineralisation of the aortic valve cells through a nuclear factor κ B/interleukin 6/bone morphogenetic protein pathway.[12] In a second study, circulating total ATX mass, and particularly ATX activity in conjunction with Lp(a) and OxPL-apoB were very strong predictors of the presence of AS, with HRs>5.[26] There are methodological differences between those studies and the current study. The current assays only measure ATX mass, but not activity, and on apoB and Lp(a) rather than whole plasma, which may explain the difference. Furthermore, the role of ATX may be more prominent within valve leaflets once OxPL are either generated or transported in by carriers such as Lp(a). Further work is needed to understand the role of lipoprotein-associated ATX versus total plasma levels and then to elucidate the potential benefice of targeting ATX over other lipid-related molecules that modulate AS progression.

Limitations

Limitations of this study include that, due to the lack of remaining plasma, ApoC-III content on apoB or apoAI containing lipoproteins could not be measured to assess their predictive value.[19] Even if there was no interaction between apoC-III and Lp(a) with age, the association of these biomarkers with faster AS progression should be assessed in an older AS population. Finally, our findings consistently support the association of high Lp(a) and apoC-III plasma levels, that was observed in a group of 22 patients, with faster progression of AS and occurrence of clinical outcomes. These data need to be confirmed in a larger group

Key questions

What is already known about this subject?

- Oxidised phospholipids (OxPL) sassociated with apolipoprotein B-100 (OxPL-apoB) and lipoprotein(a) [Lp(a)] are associated with haemodynamic progression rate of calcific aortic valve stenosis (AS) and occurrence of valve-related events, mainly defined as the need of aortic valve replacement. Other factors, such as metabolic syndrome in which triglycerides are elevated, are also associated with AS. Currently, biomarkers in the context of AS remain suboptimal to predict rapid progression and risk of events.

What does this study add?

- This study shows that apolipoprotein C-III (apoC-III), which is an important component of Lp(a), is present within diseased aortic valve leaflets. Furthermore, circulating levels of ApoC-III on Lp(a) complexes, associated with elevated Lp(a) or OxPL plasma levels, identify rapid progressors and patients with high risk of aortic valve-related events. This study supports the usefulness of these biomarkers to predict progression of AS and stratify risk of patients.

How might this impact on clinical practice?

- Identifying patients at higher risk of events or those who are prone to progress rapidly from mild-moderate to severe AS can be useful to manage and follow patients with AS. Moreover, this study provides further support to target Lp(a)/OxPL/apoC-III pathway to delay the progression of the disease using antisense oligonucleotides targeted to Lp(a) or ApoC-III, or monoclonal antibodies to OxPL.

of patients with AS, and particularly in larger number of patients with concomitant high Lp(a) and ApoC-III plasma levels.

Clinical implications

There may be potential therapeutic implications from this study, particularly following the failure of four statin trials in patients with AS.[5] These data, and the confluence of data on Lp(a)/OxPL as risk factors for aortic stenosis,[10] provide a proof of concept to design randomised clinical trials to assess the efficacy of therapies targeting Lp(a)/OxPL/apoC-III to slow AS progression. Lp(a)[27 28] and apoC-III[29] can be targeted with antisense oligonucleotides and OxPL with monoclonal antibodies such as E06.[30] In the meantime, our findings support, at least in part, the use of Lp(a) and ApoC-III as biomarkers to stratify patients with increased risk of faster AS progression and occurrence of AS-related clinical events.

CONCLUSION

ApoC-III is present in Lp(a), measured both in the proteome and by ELISA techniques. A strong interaction was noted between highest levels of ApoCIII-Lp(a) and Lp(a) or OxPL-apoB and OxPL-apo(a) in predicting rapid progression of AS, and additionally identifying fast progressors and patients at highest risk of AVR and cardiac death. These variables can be used in clinical research studies of AS and may be useful as clinical biomarkers to identify patients with the fastest rate of AS progression.

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Competing interests J-PD has served as a speaker for Abbott Laboratories, AstraZeneca, Solvay Pharma, GlaxoSmithKline, and Pfizer Canada, Inc.; has received research funding from Eli Lilly Canada; and has served on the advisory boards of Novartis, Theratechnologies, Torrent Pharmaceuticals Ltd., and Sanofi-Aventis. PM has a patent application on the use of Lp-PLA2 inhibitors in the treatment of AS. BA has received research funding from Ionis Pharmaceuticals, Merck and Pfizer. ST and JLW are co-inventors and receive royalties from patents owned by UCSD on oxidation-specific antibodies and of biomarkers related to oxidised lipoproteins and are co-founders of Oxitope, Inc. ST is a consultant to Boston Heart Diagnostics, a co-founder of Oxitope, Inc. and has a dual appointment at UCSD and Ionis Pharmaceuticals. JLW is a consultant to Ionis Pharmaceuticals and a co-founder of Oxitope, Inc.

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