Internal and Emergency Medicine (2019) 14:45–50
https://doi.org/10.1007/s11739-018-1925-8

IM - ORIGINAL

Role of lipoprotein (a) and *LPA* KIV2 repeat polymorphism in bicuspid aortic valve stenosis and calcification: a proof of concept study

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Received: 6 June 2018 / Accepted: 5 August 2018 / Published online: 11 August 2018

Abstract

Hemodynamic valvular impairment is a frequent determinant of the natural history of bicuspid aortic valve (BAV). The role of elevated Lp(a) levels and *LPA* Kringle IV type 2 (KIV-2) size polymorphism in influencing aortic valve calcification and stenosis development in patients with tricuspid aortic valve was recognized. In this study, we investigate the association between Lp(a) and *LPA* KIV-2 repeat number, and the presence of calcification and stenosis in BAV patients. Sixty-nine patients [79.7% males; median age 45(30–53) yrs], consecutively referred to Center for Cardiovascular Diagnosis or Referral Center for Marfan syndrome or related disorders, AOU Careggi, from June to November 2014, were investigated. For each patient, clinical (ECG and echocardiography) and laboratory [Lp(a) (Immunoturbidimetric assay) and *LPA* KIV-2 repeat number (real-time PCR)] evaluation were performed. Patients were compared with 69 control subjects. No significant association between Lp(a) circulating levels and *LPA* KIV-2 repeat number and BAV was evidenced. Among BAV patients, significantly higher Lp(a) levels according to calcification degree were found [no calcifications:78(42–159) mg/L, mild/moderate: 134(69–189) mg/L; severe: 560(286–1511) mg/L, $p=0.008$]. Conversely, lower *LPA* KIV-2 repeat numbers in subjects with more severe calcification degree were observed. Furthermore, higher Lp(a) levels in patients with aortic stenosis [214(67–501) mg/L vs 104(56–169) mg/L, $p=0.043$] were also found. In conclusion, present data suggest the potential role for Lp(a) as a possible risk marker useful to stratify, among BAV patients, those with a higher chance to develop valvular calcifications and aortic stenosis.

Keywords Lipoprotein (a) · Kringle IV type 2 · Bicuspid aortic valve · Calcification · Stenosis

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Introduction

Bicuspid aortic valve (BAV) represents the most common congenital heart defect, as it affects 0.5–2% of the general population [1, 2]. Hemodynamic valvular impairment is a frequent determinant of the natural history of BAV [3]. An increased risk of valvular heart disease, such as aortic valve stenosis (AVS) and regurgitation, as well as aortopathy, has been documented in BAV patients [4]. AVS occurs earlier in BAV patients than in those with tricuspid aortic valve (TAV), and the presence and severity of calcification contribute to worsen the clinical outcome [2, 5]. The role of lipid infiltration in mediating the pathophysiological mechanisms underlying AVS in TAV patients has been widely recognized [6], thus supporting the evidence of a role for risk factors shared with the atherosclerotic process in promoting this condition [7–10]. Among lipid components, several data also suggest the contribution of high levels of lipoprotein (a) [Lp(a)], acting as the major carrier of oxidized phospholipids (OxPLs), in affecting aortic valve disease [11–13]. Lp(a), might also bind, especially under conditions of excess in plasma, aortic valve endothelium, damaged by continuous mechanical stress related to the cardiac cycle, as well as high pressure and shear stress [14]. Increased Lp(a)-mediated cholesterol delivery to the valve leaflets may determine the formation of cholesterol microcrystals able to represent a source of calcification [15]. Moreover, Lp(a), via OxPLs, determines increased oxidative stress, which determines osteogenic differentiation of the endothelial cells, thus further contributing to valve calcification [16]. Lp(a) levels are under a strict genetic control. In particular, a copy number variation (CNV) in apolipoprotein (a) (*LPA*) gene (OMIM 152200)—consisting in a variable number of a 5.6 kb repeat including the exons 4 and 5 occurring between two and more than 40 times per allele—represents the main genetic determinant. This size polymorphism reflects the variability in number of repetitive copies of kringle (K) IV type 2 (KIV-2) protein domains, affecting the final dimension of the expressed protein, which inversely correlates with circulating Lp(a) serum levels [17]. To date, few data regarding the relationship between high Lp(a) levels and the presence of calcifications in BAV patients are available [18]. Therefore, based on previously reported information, in this study we investigate the association between Lp(a) plasma levels, *LPA* KIV-2 size polymorphism, reflecting a variable number of kringle (K) IV type 2 apolipoprotein (a) domains and inversely correlating with circulating Lp(a) levels [17], and the presence of calcification and stenosis in BAV patients.

Methods

Study population

Sixty-nine patients with BAV, consecutively referred to the Center for Cardiovascular Diagnosis or to the Referring Center for Marfan syndrome or related disorders, AOU Careggi, from June to November 2014, were investigated (Table 1).

For each patient, clinical history was collected and the cardiovascular risk profile was evaluated. In particular, the presence of classical cardiovascular risk factors or histories of diabetes mellitus, hypertension, dyslipidemia, smoking habit were reported. We also performed a physical examination, electrocardiogram (ECG) and echocardiogram.

At echocardiographic evaluation, BAV was diagnosed when only two cups were unequivocally identified in systole and diastole in the short axis view with a clear "fishmouth" appearance during systole as previously described [1, 19].

Table 1 Demographic and clinical characteristics of patients with bicuspid aortic valve and controls

Characteristics	BAV patients ($N=69$)	Controls ($N=69$)	p
Age, years*	45 (30–53)	52 (50–54)	<0.0001
Sex (male), *N* (%)	55 (79.7)	55 (79.7)	1.000
Smoking habit, *N* (%)	7 (10.1)	36 (52.2)	<0.0001
Diabetes, *N* (%)	2 (2.9)	5 (7.2)	0.245
Hypertension, *N* (%)	22 (31.9)	19 (27.5)	0.576
Dyslipidemia, *N* (%)	12 (17.4)	27 (39.1)	0.005
BAV types			
RL, *N*(%)	60 (87.0)	–	
RN, *N*(%)	9 (13.0)	–	
Raphe, *N*(%)	43 (62.3)	–	
BAV calcification degree			
No calcifications, *N*(%)	39 (56.5)	–	
Mild calcifications, *N*(%)	19 (27.5)	–	
Moderate calcifications, *N*(%)	7 (10.2)	–	
Severe calcifications, *N*(%)	4 (5.8)	–	
BAV stenosis degree			
No stenosis, *N*(%)	55 (79.7)	–	
Mild stenosis, *N*(%)	3 (4.4)	–	
Moderate stenosis, *N*(%)	6 (8.7)	–	
Severe stenosis, *N*(%)	5 (7.2)	–	
Pharmacological therapies			
Statins, *N*(%)	6 (8.7)	8 (11.6)	0.573
Antiplatelets, *N*(%)	2 (2.9)	3 (4.3)	0.649
Antihypertensives, *N*(%)	26 (37.7)	18 (26.1)	0.144

BAV bicuspid aortic valve

*Median (interquartile range)

Categorization of valve morphotype was also assessed (BAV_RL: right and left coronary cusps fusion, BAV-RN: right coronary and non-coronary cusps fusion and BAV-LN: left coronary and non-coronary cusps fusion). Aortic dimensions were assessed at end-diastole in the parasternal long-axis view at four levels by the leading edge method [1, 19, 20]. Aortic or mitral regurgitation was evaluated and graded by multiple criteria combining color Doppler and continuous wave Doppler signals, and aortic valve stenosis was evaluated and graded by peak aortic valve velocity [1]. Echocardiographic assessment of the aortic valve calcification degree (no calcification, mild calcification, moderate calcification and severe calcification) was also performed [21].

Sixty-nine healthy control subjects, partners or friends of the whole population of patients were enrolled. A detailed clinical evaluation of personal/familial clinical history aimed to exclude calcific valvular heart disease, and to evaluate the presence/absence of traditional cardiovascular risk factors, was also performed by an expert physician.

Both patients and controls were of Caucasian descent, drawn from the same geographical area, and unrelated to each other. All of them gave their written informed consent and the study was approved by the Institutional Review Board.

Lp(a) serum measurement

Blood withdrawal from the antecubital vein into evacuated plastic tubes (Vacutainer) without anticoagulant was performed in the morning, after overnight fasting. To determine Lp(a) concentrations, serum samples were obtained by centrifuging Vacutainer tubes at room temperature ($2000g \times 15$ min) and stored at − 80 °C until use. Lp(a) levels were measured through Randox Immunoturbidimetric Assay (Randox, Antrim, UK).

Genetic analysis

Genomic DNA was extracted from peripheral venous blood, drawn into Vacutainer K_2 EDTA tubes, using FlexiGene Kit (Qiagen, Germany).

The *LPA* KIV-2 size polymorphism was genotyped by real-time polymerase chain reaction (PCR) analysis using the 7900HT Sequence Detection System (Life Technologies) according to a previously described protocol [22]. Taqman telomerase reverse transcriptase (*TERT*) control reagent was used as a single-copy reference gene. In the frame of qPCR data normalization and analysis, C27 sample, previously predicted to have 27 *LPA* KIV-2 copies, was used as calibrator. Genotyping resulted in an estimate of the total number (sum of repeats on both alleles) of KIV-2 repeats.

Statistical analysis

Statistical analysis has been performed by SPSS package v19 (SPSS Inc., Chicago, IL, USA). Categorical variables are expressed as frequencies and percentages, whereas continuous data are given as median and interquartile (IQ) range, unless otherwise specified. The χ^2 test has been used to compare dichotomous data. As concerns continuous variables, data comparisons were performed by the non-parametric Mann–Whitney test or Kruskal–Wallis test for unpaired data, as appropriate. Correlation analyses have been performed using the non-parametric Spearman's correlation test. Logistic regression analysis, adjusted for age, hypertension, diabetes mellitus, dyslipidemia, and smoking habit have been used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for the risk of BAV calcification/stenosis according to Lp(a) levels or *LPA* KIV-2 repeat number. Statistical significance was accepted at p value < 0.05.

Results

Demographic and clinical characteristics of the study population are shown in Table 1.

BAV patients were gender-matched with the control group; median age was lower among patients in comparison to controls. The cardiovascular risk factors profile was comparable between the two groups, except for smoking habit and dyslipidemia. No significant differences in therapies management between the two groups were present. No patient has heart failure at baseline among those with or without aortic stenosis.

Among patients, 60 (87.0%) were BAV-RL and 9 (13.0%) BAV-RN. Forty-three patients (62.3%) had a raphe. In 39 patients, no valvular calcifications were observed, among the remaining patients, 19 (27.5%) exhibited mild, 7 (10.2%) moderate, and 4 (5.8%) severe calcifications (Table 1). As concerns the presence of aortic valve stenosis (AVS), mild AVS was observed in 3 (4.4%), moderate AVS in 6 (8.7%), and severe AVS in 5 (7.2%) patients (Table 1).

In the study population, *LPA* KIV-2 repeat number significantly and inversely correlated with Lp(a) levels ($r = -0.219$, $p = 0.01$).

BAV patients did not show significantly different Lp(a) circulating levels with respect to controls [108 (58–190) mg/L vs 126 (51–346), $p = 0.487$]; as concerns *LPA* KIV-2 size polymorphism, a significantly higher repeat number was observed in the patients group [17 (12–30) vs 14 (11–23), $p = 0.030$)]. Nevertheless, no significant association between *LPA* KIV-2 repeat number and BAV at logistic regression univariate analysis was observed [OR (95% CI) 1.019 (0.998–1.041), $p = 0.077$].

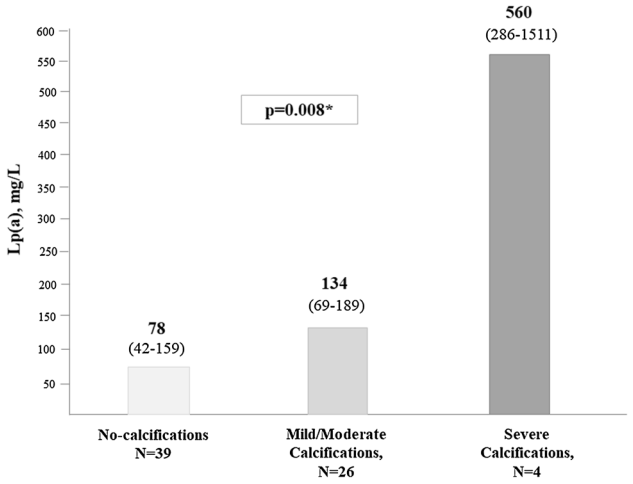

Fig. 1 Lp(a) circulating levels in BAV patients according to the calcification degree. Lp(a) values in figure are expressed as median (interquartile range). The mean ± standard deviation values are: no-calcifications 173 ± 302 mg/L, mild/moderate calcifications 227 ± 354 mg/L, severe calcifications 786 ± 690 mg/L. *Kruskal–Wallis test

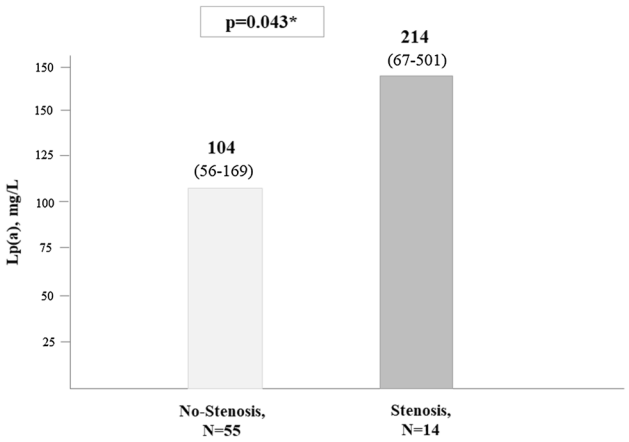

Fig. 2 Lp(a) circulating levels in BAV patients according to presence (all degrees) or absence of aortic valve stenosis. Lp(a) values in figure are expressed as median (interquartile range). The mean ± standard deviation values are: no-stenosis 190 ± 336 mg/L, stenosis 382 ± 469 mg/L. *Mann–Whitney test

Table 2 Multivariate regression analysis for association between Lp(a) and aortic calcification

Variable	OR	(95% CI)	*p*
Lp(a)	1.002	1.000–1.004	0. 085
Age	1.103	1.044–1.165	< 0.0001
Dyslipidemia	3.682	0.678–19.983	0.131
Diabetes*	–	–	–
Hypertension	0.337	0.091–1.244	0.103
Smoking habit	0.977	0.112–8.531	0.983

*No diabetic patients were present among patients without calcifications

Table 3 Multivariate regression analysis for association between Lp(a) and aortic stenosis

Variable	OR	(95% CI)	*p*
Lp(a)	1.001	1.000–1.003	0.096
Age	1.029	0.981–1.080	0.239
Dyslipidemia	1.213	0.223–6.602	0.823
Diabetes	0.563	0.024–13.389	0.722
Hypertension	0.608	0.154–2.406	0.479
Smoking habit	0.957	0.096–9.489	0.970

Risk for all degrees aortic stenosis

Among BAV patients, significant increased circulating values of Lp(a) were related to the presence and severity of aortic calcification (Fig. 1). Consistently, progressively decreased *LPA* KIV-2 repeat number according to calcification degree was observed, even if it did not reach statistical significance [no calcification: 19 (13–30), mild/moderate calcification: 14 (11–33), severe calcification: 12.5 (12–13), $p = 0.102$]. At the multivariate logistic regression analysis, after adjustment for age and traditional cardiovascular risk factors, a trend toward significance in association between Lp(a) levels and the presence of calcifications was found, even if the age remained the stronger significant determinant in the model (Table 2).

Higher Lp(a) levels were also documented in BAV patients with all degrees of aortic valve stenosis than in patients without stenosis [214 (67–501) vs 104 (56–169) mg/L, $p = 0.043$] (Fig. 2). No significant difference between BAV patients with and without aortic valve stenosis in *LPA* KIV-2 repeat number was observed [16 (12–29) mg/L vs 18 (12–30), $p = 0.922$]. At the multivariate logistic regression analysis, a trend toward significance in association between Lp(a) levels and the presence of stenosis was evidenced (Table 3).

No correlation between Lp(a) levels and root or ascending thoracic aorta diameters was observed (data not shown).

Discussion

In the present study, we document the relationship between Lp(a) levels, *LPA* KIV-2 repeat number, and the presence of calcification and valve stenosis in BAV patients. In particular, higher Lp(a) levels and lower KIV-2 repeat number were detected in BAV patients showing a higher degree of calcification. Moreover, significantly higher Lp(a) concentration, associated with a lower *LPA* KIV-2 repeat number, was recognized in BAVs showing aortic valve stenosis.

Actually, plasma Lp(a) concentration is a trait largely controlled by the *LPA* locus on chromosome 6q27, thus being less effectively modulated by diet, exercise, and environmental conditions [18]. *LPA* KIV-2 size polymorphism represents the main genetic determinant of Lp(a) levels. Due to the extremely variable KIV-2 repeat number, a broad distribution of Lp(a) levels across populations is present [18]. Data from the present study concerning median Lp(a) circulating values and KIV-2 repeat number in the control population are consistent with those observed in previous studies carried out on italian populations [22, 23].

Furthermore, our results provide novel evidence of a lack of relationship between Lp(a) and the presence of BAV per se.

Previous epidemiological data from the literature show the association between high Lp(a) levels and aortic valve calcification [7, 11, 24–26], thus highlighting the contribution of the alteration of lipid profile in the calcification process. In a large cohort of subjects, high Lp(a) levels have also been independently associated with both the presence of subclinical calcific aortic valve disease (CAVD) and the degree of calcification [27]. Moreover, data from a large Danish prospective general population studies (the Copenhagen City Heart Study and the Copenhagen General Population Study) show that elevated Lp(a) levels, associated with a low number KIV-2 repeat number, significantly influence both aortic valve stenosis and calcification [12, 28].

The possible contribution of Lp(a) in modulating both aortic valve calcification and stenosis development in BAV patients might be supported by some data. Atherosclerosis has been suggested to involve the oxidation of low-density lipoprotein-associated polyunsaturated fatty acids. Many aldehydes are formed as a result of decomposition of lipid peroxides and contribute to the development of calcification.

The contribution of Lp(a), through its intimal deposition and its effect as a major carrier of oxidized phospholipids, in determining a substrate prone to atherosclerosis progression and calcification has been widely discussed [13, 29]. Moreover, elevated Lp(a) levels, retained at areas of mechanical injury of the aortic valve, have been also suggested to contribute to the aortic valve disease development [14].

Only an observation study from Ker et al. [17], performed on ten patients, aimed to correlate Lp(a) levels with the presence or absence of aortic valve calcification in BAV patients. Data from our study are consistent with those from Ker et al. as we provide evidence of higher Lp(a) levels in BAV patients with aortic calcification. In addition to the data from Ker [17], present data provide further evidence of a contribution of higher Lp(a) levels to the degree of aortic calcification and to the development of aortic stenosis. The demonstration of the effect of PCSK9 inhibitors in reducing Lp(a) levels [30] may suggest their potential role in BAV patient treatment.

The small sample size and the lack of quantification of calcification degree through computed tomography imaging represent the main limitations of the present study.

In conclusion, our data focus attention on the possible role of Lp(a) in influencing the development of calcifications and in modulating the risk of aortic stenosis in BAV patients, thus supporting previously reported data showing the contribution of this lipid particle in the pathophysiological mechanisms responsible for CAVD. Based on previous reported observations, our data prompt the need for further studies performed in larger cohorts to establish the real effect of Lp(a) in modulating aortic valve disease. Moreover, due to the strict genetic control of Lp(a) levels, the contribution of the genetic variants responsible for modulation of Lp(a) levels might be taken into account in framing the susceptibility profile of these patients.

Funding This research was supported by departmental funds (ex-60%) for scientific research 2016 and 2017 to Prof. Betti Giusti.

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

Statement of human rights All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed consent Written informed consent was obtained from all individual participants included in the study.

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