Lipoprotein(a) Levels Are Associated With Subclinical Calcific Aortic Valve Disease in White and Black Individuals

The Multi-Ethnic Study of Atherosclerosis

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Objective—Lipoprotein(a) [Lp(a)] is a risk factor for calcific aortic valve disease (CAVD) but has not been evaluated across multiple races/ethnicities. This study aimed to determine whether Lp(a) cutoff values used in clinical laboratories to assess risk of cardiovascular disease identify subclinical CAVD and its severity and whether significant relations are observed across race/ethnicity.

Approach and Results—Lp(a) concentrations were measured using a turbidimetric immunoassay, and subclinical CAVD was measured by quantifying aortic valve calcification (AVC) through computed tomographic scanning in 4678 participants of the Multi-Ethnic Study of Atherosclerosis. Relative risk and ordered logistic regression analysis determined cross-sectional associations of Lp(a) with AVC and its severity, respectively. The conventional 30 mg/dL Lp(a) clinical cutoff was associated with AVC in white (relative risk: 1.56; confidence interval: 1.24–1.96) and was borderline significant (P=0.059) in black study participants (relative risk: 1.55; confidence interval: 0.98–2.44). Whites with levels ≥50 mg/dL also showed higher prevalence of AVC (relative risk: 1.72; confidence interval: 1.36–2.17) than those below this level. Significant associations were observed between Lp(a) and degree of AVC in both white and black individuals. The presence of existing coronary artery calcification did not affect these associations of Lp(a) and CAVD. There were no significant findings in Hispanics or Chinese.

Conclusions—Lp(a) cutoff values that are currently used to assess cardiovascular risk seem to be applicable to CAVD, but our results suggest race/ethnicity may be important in cutoff selection. Further studies are warranted to determine whether race/ethnicity influences Lp(a) and risk of CAVD incidence and its progression. **(*Arterioscler Thromb Vasc Biol*. 2016;36:1003-1009. DOI: 10.1161/ATVBAHA.115.306683.)**

Key Words: aortic valve, calcification of ■ atherosclerosis ■ immunoassay ■ prevalence ■ risk factor

Calcific aortic valve disease (CAVD) is a progressive disorder that encompasses a spectrum of valve pathologies ranging from calcification of valve leaflets to obstruction of blood outflow. Early subclinical stages of CAVD are characterized by aortic valve calcification (AVC), which has historically been considered a benign degenerative condition that occurs with advancing age but is now recognized as a risk factor for cardiovascular disease. Indeed, AVC has been shown to independently predict cardiovascular events,[1] increase risk of fatal coronary heart disease (CHD),[2] and may progress to valve stenosis—a stiffening or narrowing of the aortic valve and most common cause of valve replacement.[3] Several factors have been identified that promote CAVD development that are largely shared with CHD including, but not limited to, age, sex, hypertension, smoking, type II diabetes, hypercholesterolemia,[4–7] and, more recently, elevated concentrations of lipoprotein (a) [Lp(a)].[7,8]

See accompanying editorial on page 774

Lp(a) particles are a subclass of low-density lipoproteins (LDL) primarily distinguished by their apolipoprotein(a) component. Similar to conventional LDL, elevated Lp(a) levels are an established independent risk factor for CHD as reported by case–control and prospective studies.[9–11] By comparison, evidence relating Lp(a) to CAVD and other valve disorders is less abundant, albeit consistent. Prospective and cross-sectional studies have reported positive associations of Lp(a) with both early and later stages of CAVD,[12–16] and Mendelian randomization studies indicate that Lp(a) directly contributes to disease[12,15]; however, there are critical aspects yet to be examined. First, race-based differences in median Lp(a) levels have been well documented with black individuals typically showing 2- to 3-fold higher levels compared with whites or Hispanics.[17–19]

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Nonstandard Abbreviations and Acronyms

AVC	aortic valve calcification
CAC	coronary artery calcium
CHD	coronary heart disease
CAVD	calcific aortic valve disease
CI	confidence interval
Lp(a)	lipoprotein(a)
LDL	low-density lipoproteins
MESA	multi-ethnic study of atherosclerosis
OR	odds ratio
RR	relative risk

Remarkably, these higher Lp(a) levels in black individuals do not translate to a corresponding 2- to 3-fold higher risk of Lp(a)-associated disease—as shown in studies of CHD.[18,19] Whether this phenomenon is evident in Lp(a)-associated CAVD or degree of calcification is unknown, but race/ethnicity may modify whether Lp(a) confers risk of CAVD.

In addition to a possible race/ethnicity-related modification of Lp(a) and valve disease, it remains unknown whether Lp(a) cutoff values used in clinical laboratories to assess cardiovascular risk (30 and 50 mg/dL) may be used in the context of CAVD. Notably, both the 30 and 50 mg/dL Lp(a) cutoffs have been shown to confer higher risk of CHD in black individuals, whereas only the 50 mg/dL cutoff was shown to associate with higher disease risk in whites and Hispanics[18]—whether this phenomenon is also found in prevalent CAVD is unknown and is critical information for clinical laboratories. In this analysis, we examined whether elevated levels of Lp(a) are related to the presence of subclinical CAVD and degree of AVC among 1347 black, 1708 white, 1064 Hispanic, and 559 Chinese American participants of the Multi-Ethnic Study of Atherosclerosis (MESA). In addition to conventional risk factors, the presence of existing subclinical atherosclerosis as determined by coronary artery calcium (CAC), and serum phosphate levels were included as covariates.

Materials and Methods

Materials and Methods are available in the online-only Data Supplement.

Results

Sample Characteristics

Characteristics of MESA participants at baseline are shown in Table 1. Age and sex distributions were comparable. Chinese Americans had the lowest percentage of smokers and hypertensive participants, whereas whites had the fewest diabetic participants. Blacks had higher prevalence of hypertension, lower levels of triglycerides, and significantly higher levels of Lp(a) compared with other groups. Whites had the highest prevalence (14.5%) of subclinical CAVD as assessed by AVC, whereas Chinese Americans had the lowest (6.6%). Whites showed the most severe AVC cases with 93 (5.4%) individuals having an AVC score of >100, whereas the Chinese Americans had the fewest cases with 9 individuals (1.6%).

Continuous Lp(a) and Prevalence of Subclinical CAVD

Associations between log-transformed Lp(a) levels and the presence of AVC are shown in Table 2. A significant association

Table 1. Characteristics of MESA Participants in 4 Race/Ethnic Groups at Visit 1

	Blacks	White	Hispanics	Chinese
N	1347	1708	1064	559
Age (yr)	61 (52–70)	62 (54–71)	61 (52–69)	62 (53–71)
Sex (male)	621 (46.1%)	813 (47.6%)	517 (48.6%)	217 (38.8%)
Smoker (former or current)	726 (53.9%)	929 (54.4%)	504 (47.4%)	137 (24.5%)
Diabetic or on diabetes mellitus meds	196 (14.6%)	86 (5.0%)	171 (16.1%)	55 (9.8%)
Hypertensive	428 (31.8%)	325 (19.0%)	257 (24.2%)	126 (22.5%)
On hypertension meds	613 (45.5%)	493 (28.8%)	305 (28.7%)	138 (24.7%)
Non-Lp(a) LDL-C (mg/dL)	113 (92–133)	115 (97–136)	116 (97–137)	114 (96–132)
HDL-C (nmol/L)	1.29 (1.06–1.57)	1.29 (1.06–1.60)	1.16 (0.98–1.40) *	1.24 (1.03–1.50) *
Triglycerides (nmol/L)	1.00 (0.75–1.38)*	1.24 (0.85–1.81) *	1.50 (1.06–2.13) *	1.37 (0.96–1.91) *
Lp(a) (mg/dL)	35.1 (20.4–61.6)*	13.0 (5.8–29.6)	13.1 (6.3–28.8)	12.9 (7.7–23.4)
AVC presence	157 (11.7%)	248 (14.5%)	140 (13.2%)	37 (6.6%)
AVC severity (Agatston units)				
0	1190	1460	924	522
>0 to 100	101	155	81	28
>100	56	93	59	9

Data are shown in median (interquartile range) for continuous variable and as count (%) for categorical variable. Definition: smoker (former and current), diabetic (treated and untreated), and hypertensive (systolic blood pressure ≥140 mm Hg). AVC indicates aortic valve calcification; HDL, high-density lipoproteins; LDL, low-density lipoproteins; and Lp(a), lipoprotein(a).

*$P<0.05$ indicating significant difference compared with other race/ethnicity groups.

Table 2. Association of Lp(a) Levels With the Presence of Subclinical Calcific Aortic Valve Disease

	Blacks	Whites	Hispanics	Chinese Americans	All Groups
N	1324	1677	1044	548	4593*
Per log unit					
Estimated RR	1.26	1.19†	0.94	0.91	1.11†
95% CI	0.97–1.65	1.06–1.33†	0.85–1.03	0.23–3.64	1.02–1.21†
P value	0.088	0.0023†	0.18	0.90	0.021†
≥30 mg/dL					
N (%)‡	774 (57.5)	423 (24.8)	258 (24.2)	108 (19.3)	1563 (33.4)
Estimated RR	1.55	1.56†	1.09	2.18	1.38†
95% CI	0.98–2.44	1.24–1.96†	0.79–1.51	0.52–9.21	1.18–1.62†
P value	0.059	<0.001†	0.61	0.29	<0.001†
≥50 mg/dL					
N (%)§	445 (33.0)	255 (14.9)	140 (13.2)	54 (9.7)	894 (19.1)
Estimated RR	1.24	1.72†	1.24	2.25	1.44†
95% CI	0.85–1.80	1.36–2.17†	0.82–1.87	0.54–9.44	1.21–1.72†
P value	0.26	<0.001†	0.31	0.27	<0.001†

RR (95% CI, *P* value) is presented per unit increment in log Lp(a) or categorically (30 or 50 mg/dL). Models were adjusted for age, sex, hypertension (systolic blood pressure and medication), smoking, education status, diabetes mellitus, non-Lp(a)-LDL-C, HDL-C, log(triglycerides), presence of coronary artery calcium, and serum phosphate levels. *P*<0.05 indicates significant associations. CI indicates confidence interval; HDL, high-density lipoproteins; LDL, low-density lipoproteins; Lp(a), lipoprotein(a); and RR, Relative risk.

*Excluding individuals with missing covariate data.

†*P*<0.05.

‡Number of individuals with Lp(a) ≥ 30 mg/dL.

§Number of individuals with Lp(a) ≥ 50 mg/dL.

[relative risk (RR)=1.11; 95% confidence interval (CI): 1.02–1.21; *P*=0.02] was observed in the entire sample after adjusting for covariates including age, sex, systolic blood pressure, taking hypertension medication, smoking, education, diabetes mellitus, non–Lp(a)-LDL-C, high-density lipoprotein-C, triglycerides (log-transformed), serum phosphate levels, and the presence of CAC. When stratified by race/ethnicity, the association between Lp(a) and AVC remained significant in white participants (RR=1.19; 95% CI: 1.06–1.33; *P*=0.0023). No significant associations were observed in Hispanics or Chinese Americans but approached significance in black participants (RR=1.26; 95% CI: 0.97–1.65; *P*=0.088). A formal interaction test suggested that the association of Lp(a) (per log unit) and the presence of AVC varies dependent on race/ethnicity ($P_{interaction}$=0.03).

Lp(a) Cutoffs and Prevalence of Subclinical CAVD

Lp(a) cutoff values were next evaluated to determine whether they differentially associated with the presence of AVC across races. The 30 mg/dL cutoff identified higher prevalence of AVC in white individuals (RR=1.56; 95% CI: 1.24–1.96; *P*<0.001) compared with those below 30 mg/dL. This relationship was borderline significant in black study participants (RR: 1.55; CI: 0.98–2.44; *P*=0.059). The 50 mg/dL cutoff identified higher prevalence of AVC in white MESA participants (RR=1.72; 95% CI: 1.36–2.17; *P*<0.001) but was not significant in black participants (RR=1.24; 95% CI: 0.85–1.85; *P*=0.26). No significant associations were observed in Hispanics or Chinese Americans for either cutoff value.

Lp(a) and AVC Severity

Associations of Lp(a) and the degree of calcification on the aortic valve were examined as above, testing Lp(a) as a continuous or categorical variable (Table 3) with identical covariate adjustments; however, odds ratios (OR) were generated from ordered logistic regression in place of using a RR regression approach. Lp(a) (per 1 log unit) was associated with the severity of AVC in black (OR = 1.48; 95% CI: 1.18–1.87) and white participants (OR = 1.33; 95% CI: 1.17–1.51). When examined using either 30 or 50 mg/dL dichotomizations, results were similar to the above. White individuals showed a greater likelihood of more severe AVC when Lp(a) exceeded 30 mg/dL (OR: 2.22; 95% CI: 1.59–3.10) or 50 mg/dL (OR: 2.95; 95% CI: 2.03–4.29). Likewise, black individuals showed a greater likelihood of more severe AVC when Lp(a) exceeded 30 mg/dL (OR: 1.93; CI: 1.29–2.91) or 50 mg/dL (OR: 1.71; CI: 1.17–2.50). No significant associations were observed in Chinese or Hispanic subpopulations examining Lp(a) as a continuous variable or using either cutoff value; however, associations approached significance using the 50 mg/dL cutoff in both Chinese (*P*=0.087) and Hispanic study participants (*P*=0.062).

Existing Atherosclerosis and Serum Phosphate

Additional covariates were included in the above models that have been suggested to influence CAVD—specifically, levels of serum phosphate as well as the presence of atherosclerosis as estimated by CAC. Serum phosphate levels were weakly correlated with Lp(a) in BLACK (corr=0.099; *P*<0.001) and

Table 3. Association of Lp(a) Levels and Severity of Aortic Valve Calcification

	Blacks	Whites	Hispanics	Chinese Americans	All Groups
N	1324	1677	1044	548	4593*
per log unit					
Estimated OR	1.48†	1.33†	1.01	0.97	1.21†
95% CI	1.18–1.87†	1.17–1.51†	0.87–1.17	0.66–1.43	1.11–1.31†
P value	<0.001†	<0.001†	0.91	0.87	<0.001†
≥30 mg/dL					
N (%)‡	774 (57.5)	423 (24.8)	258 (24.2)	108 (19.3)	1563 (33.4)
Estimated OR	1.93†	2.22†	1.37	1.14	1.80†
95% CI	1.29–2.91†	1.59–3.10†	0.86–2.17	0.44–2.91	1.46–2.23†
P value	0.001†	<0.001†	0.19	0.79	<0.001†
≥50 mg/dL					
N (%)§	445 (33.0)	255 (14.9)	140 (13.2)	54 (9.7)	894 (19.1)
Estimated OR	1.71†	2.95†	3.01	1.65	2.14†
95% CI	1.17–2.50†	2.03–4.29†	0.94–9.58	0.93–2.92	1.69–2.71†
P value	0.005†	<0.001†	0.062	0.087	<0.001†

Lp(a) and severity of AVC (categorized by Agatston scores of 0, 1-100, and >100) are shown below (estimated OR, 95% CI, *P* value). Models were adjusted for age, sex, hypertension (systolic blood pressure and medication), smoking, education status, diabetes mellitus, non-Lp(a)-LDL-C, HDL-C, log(triglycerides), presence of coronary artery calcium, and serum phosphate levels. *P*<0.05 indicates significant associations. AVC indicates aortic valve calcification; CI, confidence interval; HDL, high-density lipoproteins; LDL, low-density lipoproteins; Lp(a), lipoprotein(a); and OR, odds ratio.

*Excluding individuals with missing covariate data.

†*P*<0.05.

‡Number of individuals with Lp(a) ≥30 mg/dL.

§Number of individuals with Lp(a) ≥50 mg/dL.

white participants (corr=0.059; *P*=0.02). Serum phosphate directly correlated with AVC in black individuals (corr=0.010; *P*<0.001) but was inversely correlated in whites (corr=−0.04; *P*<0.001). Direct correlations of serum phosphate with the exposure [Lp(a)] and outcome variables (AVC) in black participants (but not in whites) attenuated the associations of Lp(a) and AVC in this subgroup up including it as a covariate.

In contrast, CAC was only associated with AVC in the subcohort using a regression model and adjusting for age, sex, education, diabetes mellitus, systolic blood pressure, hypertension meds, smoking, LDL, high-density lipoprotein, and triglycerides (RR=1.71; *P*<0.001). CAC was not associated with Lp(a) in the MESA data set, and the inclusion of CAC into statistical models did not appreciably influence relations of Lp(a) and AVC in the subcohort or among races/ethnicities.

Discussion

In a subcohort of 4678 MESA participants, higher Lp(a) levels were associated with the presence of subclinical CAVD and degree of valve calcification independent of age, sex, hypertension, smoking, education, diabetes mellitus, non–Lp(a)-LDL-C, high-density lipoprotein-C, triglycerides, serum phosphate, and existing CAC with a significant race interaction. Applying Lp(a) cutoffs that are currently used in clinical laboratories to evaluate cardiovascular risk showed that white participants with levels exceeding 30 mg/dL had a higher prevalence of AVC and higher likelihood of more severe AVC than those below this level. Similarly, this cutoff value revealed a borderline significant relation with AVC (*P*=0.059) and more severe AVC in black individuals. The 50 mg/dL cutoff identified higher prevalence of AVC in white participants alone but was associated with more severe valve calcification in both black and white individuals.

Lp(a) and Aortic Valve Disease

Circulating concentrations of Lp(a) are largely determined by the apolipoprotein(a)-encoding *LPA* gene,[20,21] and initial studies of Lp(a) and aortic valve-related outcomes focused on *LPA* genotypes. The first study to suggest a role of Lp(a) in CAVD development was a genome wide-association analysis conducted in 3 cohorts, including MESA. Investigators showed that the *LPA* gene variant (rs10455872) was associated with AVC in both whites and black individuals. This relationship was further shown to be mediated by circulating Lp(a) concentrations—although only the European/white population was tested.[12] Two subsequent studies in the European Prospective Investigation into Cancer-Norfolk[15] and 2 Danish cohorts[14] also showed that elevated Lp(a) levels were associated with higher risk of CAVD incidence. Finally, and most recently, a cross-sectional analysis of 129 Dutch individuals with familial hypercholesterolemia showed that +10 mg/dL increments in Lp(a) were associated with 11% greater likelihood of CAVD (OR=1.11; 95% CI = 1.01–1.20, *P*=0.03).[16] Collectively, these results indicate that higher Lp(a) levels are associated with CAVD.

The present analysis expands on previous studies by evaluating whether Lp(a) cutoff values detect the presence and severity of AVC among the 4 different races/ethnicities. In whites, our results indicate that 30 or 50 mg/dL cutoff values reveal respective 56% and 72% significantly higher prevalence of AVC (P<0.001) as well as respective 122% and 195% higher likelihood of greater valve calcification than those below these cutoffs. Given these data and overlapping CIs, either cutoff seems suitable to assess the presence or degree of AVC in whites. Based on analysis of Lp(a) as a continuous variable, higher Lp(a) levels promote higher prevalence and severity of valve disease.

Black individuals showed a more complex relation of AVC with Lp(a) than whites. The 30 mg/dL cutoff revealed a borderline significant 55% higher prevalence of AVC (P=0.059) and a 93% significantly higher likelihood of more severe valve calcification compared with black participants below this cutoff. Unexpectedly, the 50 mg/dL cutoff value revealed a nonsignificant 24% higher prevalence of AVC, but a significant 71% higher likelihood of more severe AVC (P=0.005). In terms of overall disease prevalence, black study participants had a lower prevalence of subclinical CAVD (11.7%) compared with whites (14.5%) despite having 2- to 3-fold higher median Lp(a) levels (35.1 mg/dL) versus whites (13.0 mg/dL). Based strictly on the significance values of the findings, the lower 30 mg/dL cutoff may be appropriate for black individuals for identifying CAVD risk, but further research is needed to better characterize the relation of Lp(a) with CAVD in this population—with particular focus on determining whether Black individuals are protected from their relatively high levels of Lp(a) compared with whites.

Lp(a) and AVC in Hispanics and Chinese

Null findings in Hispanic participants were not anticipated. Indeed, an association of the *LPA* gene variant (rs10455872) with subclinical CAVD was previously reported in Hispanics within the MESA population (OR=2.75; P=0.004), and it has further been shown that the *LPA* gene accounts for 40% to 90% of the variation in Lp(a) levels depending on ethnicity.[20–22] The lack of an association in Hispanic participants suggests that the genetic link between Lp(a) and valve calcification may not be mediated by plasma Lp(a) levels or there are additional modifying variables that must be considered.

In contrast to findings in Hispanics, null findings in Chinese American participants were expected based on previous findings showing inconsistent relations of Lp(a) levels with cardiovascular-related disease.[18,23,24] Indeed, it has been previously reported that Lp(a) does not associate with CHD incidence in the MESA Chinese subpopulation.[18] Despite the null finding in the present analysis, the wide CIs in this group are remarkable. Ultimately, the above null findings should be replicated in other cohorts, but these initial observations coupled with the significant race interaction (P=0.03) when Lp(a) is treated as a continuous variable, suggesting that it does not influence subclinical CAVD in Hispanics and Chinese individuals.

Lp(a) and CAC

Calcification of coronary arteries has previously been shown to associate with subclinical CAVD,[25,26] but relations among Lp(a), CAC, and CAVD have not been examined. This study confirms previous findings that individuals with CAC have a higher prevalence of CAVD (RR=1.71; P<0.001). This association likely indicates that these pathophysiological processes share risk factors or the presence of one increases the risk for developing the other. In contrast, Lp(a) was not associated with CAC in this MESA subcohort in agreement with several previous studies[27–32] although not all. Upon including CAC as a covariate in our model, the relationship between Lp(a) and CAVD was not appreciably affected, suggesting that Lp(a) and CAC are independent risk factors of CAVD. Ultimately, further prospective and longitudinal studies will be better suited for identifying relations and temporality of CAC and CAVD than is possible using the present cross-sectional design, but Lp(a) levels seem to be a risk factor for CAVD alone.

Clinical Implications in Disease Development

Subclinical CAVD may be present in 15% to 40% of adults depending on age and race/ethnicity[33] and is projected to increase with the aging population.[34] Early CAVD may advance to valve stenosis and blockage,[35] and therefore, assessing subclinical CAVD and its risk factors may identify advancement in valve disease. Although not regularly ordered by preventative cardiologists, AVC is readily available with routine chest computed tomography used for CAC detection. With respect to Lp(a), whether it is a viable clinical target or may otherwise inform clinical decisions regarding risk management of valve disease remains unclear. Lp(a) is still considered an unmodifiable lipoprotein risk factor at present, but development of Lp(a)-lowering therapies are currently underway.[36,37]

Strengths and Limitations

This study provides the first large-scale cross-sectional evaluation of Lp(a) concentrations and subclinical CAVD across 4 different races/ethnic groups. To avoid the inherent issues in accurately measuring Lp(a), mass concentrations were quantified using a latex-enhanced turbidimetric immunoassay that controls for the heterogeneous sizes of the apolipoprotein(a) component of Lp(a).[38] In terms of study limitations, the relatively few cases of subclinical CAVD in Chinese participants compared with other subpopulations limited statistical power, and null findings in Hispanic and Chinese subpopulations need to be interpreted with caution and confirmed by additional cohort studies. The cross-sectional study design prohibits the determination of temporality, but findings support a role for Lp(a) in aortic disease when coupled with other prospective analyses. Additional research using longitudinal approaches will better characterize whether high Lp(a) levels increase risk of CAVD in these different subpopulations.

Conclusions

In summary, significant associations of Lp(a) and subclinical CAVD were observed in black and white individuals in a subcohort of 4678 MESA participants. Together with the presence of a significant race interaction, race/ethnicity may influence

whether elevated levels of Lp(a) increase risk of subclinical CAVD, but further studies are warranted to determine whether Lp(a) levels increase risk of incident CAVD and its progression and whether certain races/ethnicities may be protected from the pathogenic influence of Lp(a).

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Disclosures

None.

References

1. Owens DS, Budoff MJ, Katz R, Takasu J, Shavelle DM, Carr JJ, Heckbert SR, Otto CM, Probstfield JL, Kronmal RA, O'Brien KD. Aortic valve calcium independently predicts coronary and cardiovascular events in a primary prevention population. *JACC Cardiovasc Imaging*. 2012;5:619–625. doi: 10.1016/j.jcmg.2011.12.023.
2. Otto CM, Burwash IG, Legget ME, Munt BI, Fujioka M, Healy NL, Kraft CD, Miyake-Hull CY, Schwaegler RG. Prospective study of asymptomatic valvular aortic stenosis. Clinical, echocardiographic, and exercise predictors of outcome. *Circulation*. 1997;95:2262–2270.
3. Iung B, Vahanian A. Degenerative calcific aortic stenosis: a natural history. *Heart*. 2012;98 (Suppl 4):iv7–iv13.
4. Otto CM, Lind BK, Kitzman DW, Gersh BJ, Siscovick DS. Association of aortic-valve sclerosis with cardiovascular mortality and morbidity in the elderly. *N Engl J Med*. 1999;341:142–147. doi: 10.1056/NEJM199907153410302.
5. Fox CS, Vasan RS, Parise H, Levy D, O'Donnell CJ, D'Agostino RB, Benjamin EJ; Framingham Heart Study. Mitral annular calcification predicts cardiovascular morbidity and mortality: the Framingham Heart Study. *Circulation*. 2003;107:1492–1496.
6. Bella JN, Tang W, Kraja A, Rao DC, Hunt SC, Miller MB, Palmieri V, Roman MJ, Kitzman DW, Oberman A, Devereux RB, Arnett DK. Genome-wide linkage mapping for valve calcification susceptibility loci in hypertensive sibships: the Hypertension Genetic Epidemiology Network Study. *Hypertension*. 2007;49:453–460. doi: 10.1161/01.HYP.0000256957.10242.75.
7. Rajamannan NM, Evans FJ, Aikawa E, Grande-Allen KJ, Demer LL, Heistad DD, Simmons CA, Masters KS, Mathieu P, O'Brien KD, Schoen FJ, Towler DA, Yoganathan AP, Otto CM. Calcific aortic valve disease: not simply a degenerative process: a review and agenda for research from the National Heart and Lung and Blood Institute Aortic Stenosis Working Group. Executive summary: Calcific aortic valve disease-2011 update. *Circulation*. 2011;124:1783–1791. doi: 10.1161/CIRCULATIONAHA.110.006767.
8. Yutzey KE, Demer LL, Body SC, Huggins GS, Towler DA, Giachelli CM, Hofmann-Bowman MA, Mortlock DP, Rogers MB, Sadeghi MM, Aikawa E. Calcific aortic valve disease: a consensus summary from the Alliance of Investigators on Calcific Aortic Valve Disease. *Arterioscler Thromb Vasc Biol*. 2014;34:2387–2393. doi: 10.1161/ATVBAHA.114.302523.
9. Clarke R, Peden JF, Hopewell JC, et al; PROCARDIS Consortium. Genetic variants associated with Lp(a) lipoprotein level and coronary disease. *N Engl J Med*. 2009;361:2518–2528. doi: 10.1056/NEJMoa0902604.
10. Erqou S, Kaptoge S, Perry PL, Di Angelantonio E, Thompson A, White IR, Marcovina SM, Collins R, Thompson SG, Danesh J, Collaboration ERF. Lipoprotein(a) concentration and the risk of coronary heart disease, stroke, and nonvascular mortality. *JAMA*. 2009;302:412–423
11. Kamstrup PR, Tybjaerg-Hansen A, Steffensen R, Nordestgaard BG. Genetically elevated lipoprotein(a) and increased risk of myocardial infarction. *JAMA*. 2009;301:2331–2339. doi: 10.1001/jama.2009.801.
12. Thanassoulis G, Campbell CY, Owens DS, et al; CHARGE Extracoronary Calcium Working Group. Genetic associations with valvular calcification and aortic stenosis. *N Engl J Med*. 2013;368:503–512. doi: 10.1056/NEJMoa1109034.
13. Langsted A, Varbo A, Kamstrup PR, Nordestgaard BG. Elevated lipoprotein(a) does not cause low-grade inflammation despite causal association with aortic valve stenosis and myocardial infarction: a study of 100,578 individuals from the general population. *J Clin Endocrinol Metab*. 2015;100:2690–2699. doi: 10.1210/jc.2015-1096.
14. Kamstrup PR, Tybjærg-Hansen A, Nordestgaard BG. Elevated lipoprotein(a) and risk of aortic valve stenosis in the general population. *J Am Coll Cardiol*. 2014;63:470–477. doi: 10.1016/j.jacc.2013.09.038.
15. Arsenault BJ, Boekholdt SM, Dubé MP, Rhéaume E, Wareham NJ, Khaw KT, Sandhu MS, Tardif JC. Lipoprotein(a) levels, genotype, and incident aortic valve stenosis: a prospective Mendelian randomization study and replication in a case-control cohort. *Circ Cardiovasc Genet*. 2014;7:304–310. doi: 10.1161/CIRCGENETICS.113.000400.
16. Vongpromek R, Bos S, Ten Kate GJ, Yahya R, Verhoeven AJ, de Feyter PJ, Kronenberg F, Roeters van Lennep JE, Sijbrands EJ, Mulder MT. Lipoprotein(a) levels are associated with aortic valve calcification in asymptomatic patients with familial hypercholesterolaemia. *J Intern Med*. 2015;278:166–173. doi: 10.1111/joim.12335.
17. Marcovina SM, Albers JJ, Wijsman E, Zhang Z, Chapman NH, Kennedy H. Differences in Lp[a] concentrations and apo[a] polymorphs between black and white Americans. *J Lipid Res*. 1996;37:2569–2585.
18. Guan W, Cao J, Steffen BT, Post WS, Stein JH, Tattersall MC, Kaufman JD, McConnell JP, Hoefner DM, Warnick R, Tsai MY. Race is a key variable in assigning lipoprotein(a) cutoff values for coronary heart disease risk assessment: the Multi-Ethnic Study of Atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2015;35:996–1001. doi: 10.1161/ATVBAHA.114.304785.
19. Virani SS, Brautbar A, Davis BC, Nambi V, Hoogeveen RC, Sharrett AR, Coresh J, Mosley TH, Morrisett JD, Catellier DJ, Folsom AR, Boerwinkle E, Ballantyne CM. Associations between lipoprotein(a) levels and cardiovascular outcomes in black and white subjects: the Atherosclerosis Risk in Communities (ARIC) Study. *Circulation*. 2012;125:241–249. doi: 10.1161/CIRCULATIONAHA.111.045120.
20. Nordestgaard BG, Chapman MJ, Ray K, et al; European Atherosclerosis Society Consensus Panel. Lipoprotein(a) as a cardiovascular risk factor: current status. *Eur Heart J*. 2010;31:2844–2853. doi: 10.1093/eurheartj/ehq386.
21. Boerwinkle E, Leffert CC, Lin J, Lackner C, Chiesa G, Hobbs HH. Apolipoprotein(a) gene accounts for greater than 90% of the variation in plasma lipoprotein(a) concentrations. *J Clin Invest*. 1992;90:52–60. doi: 10.1172/JCI115855.
22. Sandholzer C, Hallman DM, Saha N, Sigurdsson G, Lackner C, Császár A, Boerwinkle E, Utermann G. Effects of the apolipoprotein(a) size polymorphism on the lipoprotein(a) concentration in 7 ethnic groups. *Hum Genet*. 1991;86:607–614.
23. Yang WX, Yang Z, Wu YJ, Qiao SB, Yang YJ, Chen JL. Factors associated with coronary artery disease in young population (age ≤ 40): analysis with 217 cases. *Chin Med Sci J*. 2014;29:38–42.
24. Liang XH, Huang CZ. [Detection of serum Lp(a) level of coronary heart disease and its clinical significance]. *Hunan Yi Ke Da Xue Xue Bao*. 2001;26:227–228.
25. Kaplan S, Aronow WS, Lai H, Dilmanian H, Deluca AJ, Weiss MB, Belkin RN. Patients with echocardiographic aortic valve calcium or mitral annular calcium have an increased prevalence of moderate or severe coronary artery calcium diagnosed by cardiac computed tomography. *Int J Angiol*. 2007;16:45–46.
26. Wong ND, Sciammarella M, Arad Y, Miranda-Peats R, Polk D, Hachamovich R, Friedman J, Hayes S, Daniell A, Berman DS. Relation of thoracic aortic and aortic valve calcium to coronary artery calcium and risk assessment. *Am J Cardiol*. 2003;92:951–955.
27. Guerra R, Yu Z, Marcovina S, Peshock R, Cohen JC, Hobbs HH. Lipoprotein(a) and apolipoprotein(a) isoforms: no association with coronary artery calcification in the Dallas Heart Study. *Circulation*. 2005;111:1471–1479. doi: 10.1161/01.CIR.0000159263.50305.BD.
28. Nishino M, Malloy MJ, Naya-Vigne J, Russell J, Kane JP, Redberg RF. Lack of association of lipoprotein(a) levels with coronary calcium deposits in asymptomatic postmenopausal women. *J Am Coll Cardiol*. 2000;35:314–320.
29. Lee TC, O'Malley PG, Feuerstein I, Taylor AJ. The prevalence and severity of coronary artery calcification on coronary artery computed tomography in black and white subjects. *J Am Coll Cardiol*. 2003;41:39–44.

30. Taylor AJ, Feuerstein I, Wong H, Barko W, Brazaitis M, O'Malley PG. Do conventional risk factors predict subclinical coronary artery disease? Results from the Prospective Army Coronary Calcium Project. *Am Heart J*. 2001;141:463–468. doi: 10.1067/mhj.2001.113069.
31. Erbel R, Lehmann N, Churzidse S, et al; Heinz Nixdorf Recall Study Investigators. Gender-specific association of coronary artery calcium and lipoprotein parameters: the Heinz Nixdorf Recall Study. *Atherosclerosis*. 2013;229:531–540. doi: 10.1016/j.atherosclerosis.2013.04.015.
32. Kullo IJ, Bailey KR, Bielak LF, Sheedy PF 2nd, Klee GG, Kardia SL, Peyser PA, Boerwinkle E, Turner ST. Lack of association between lipoprotein(a) and coronary artery calcification in the Genetic Epidemiology Network of Arteriopathy (GENOA) study. *Mayo Clin Proc*. 2004;79:1258–1263. doi: 10.4065/79.10.1258.
33. Smith JG, Luk K, Schulz CA, et al; Cohorts for Heart and Aging Research in Genetic Epidemiology (CHARGE) Extracoronary Calcium Working Group. Association of low-density lipoprotein cholesterol-related genetic variants with aortic valve calcium and incident aortic stenosis. *JAMA*. 2014;312:1764–1771. doi: 10.1001/jama.2014.13959.
34. Thaden JJ, Nkomo VT, Enriquez-Sarano M. The global burden of aortic stenosis. *Prog Cardiovasc Dis*. 2014;56:565–571. doi: 10.1016/j.pcad.2014.02.006.
35. Nishimura RA, Otto CM, Bonow RO, Carabello BA, Erwin JP 3rd, Guyton RA, O'Gara PT, Ruiz CE, Skubas NJ, Sorajja P, Sundt TM 3rd, Thomas JD; American College of Cardiology/American Heart Association Task Force on Practice Guidelines. 2014 AHA/ACC guideline for the management of patients with valvular heart disease: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol*. 2014;63:e57–185. doi: 10.1016/j.jacc.2014.02.536.
36. Koschinsky M, Boffa M. Lipoprotein(a) as a therapeutic target in cardiovascular disease. *Expert Opin Ther Targets*. 2014;18:747–757. doi: 10.1517/14728222.2014.920326.
37. Tsimikas S, Viney NJ, Hughes SG, Singleton W, Graham MJ, Baker BF, Burkey JL, Yang Q, Marcovina SM, Geary RS, Crooke RM, Witztum JL. Antisense therapy targeting apolipoprotein(a): a randomised, double-blind, placebo-controlled phase 1 study. *Lancet*. 2015;386:1472–1483. doi: 10.1016/S0140-6736(15)61252-1.
38. Marcovina SM, Albers JJ, Scanu AM, Kennedy H, Giaculli F, Berg K, Couderc R, Dati F, Rifai N, Sakurabayashi I, Tate JR, Steinmetz A. Use of a reference material proposed by the International Federation of Clinical Chemistry and Laboratory Medicine to evaluate analytical methods for the determination of plasma lipoprotein(a). *Clin Chem*. 2000;46:1956–1967.

Significance

Lipoprotein(a) [Lp(a)] is an low-density lipoprotein (LDL) particle subclass recently found to increase risk of subclinical calcific aortic valve disease (CAVD), which may contribute to aortic valve stenosis or heart disease. Notably, there are significant race-based differences in Lp(a), and it remains unknown whether this may influence valvular disease development. In this study of 4679 study participants, higher Lp(a) was found to associate with higher prevalence of subclinical CAVD in white participants. Applying Lp(a) clinical laboratory cutoffs likewise showed that white participants with levels ≥30 or ≥50 mg/dL had a higher prevalence of CAVD and more severe aortic valve calcification, whereas both cutoffs were only associated with more severe aortic valve calcification in black study participants. No relationship between Lp(a) and subclinical CAVD was observed in Hispanics or Chinese. Taken together, race/ethnicity may be an important variable in determining whether elevated Lp(a) identifies subclinical CAVD or severity of aortic valve calcification. The present observations may help identify at-risk individuals and inform clinical decisions for disease risk management.

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Lipoprotein(a) Levels Are Associated With Subclinical Calcific Aortic Valve Disease in White and Black Individuals: The Multi-Ethnic Study of Atherosclerosis

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MATERIALS AND METHODS

Study Population

The design of the MESA study has been previously described,[1] and information about the MESA protocol is available at www.mesa-nhlbi.org. Briefly, 6814 men and women between the ages of 45 and 84 years without clinical evidence of cardiovascular disease were recruited from six communities in the US. Institutional Review Board approval was obtained at all MESA sites, and all participants gave informed consent.

The current study excluded participants who were taking lipid-lowering medication at baseline (n=1090)[2] as well as a subcohort of 1000 MESA participants where specimen volume was limited. The remaining study population of 4,678 individuals was composed of the following race/ethnicities: Black (n=1,347), Caucasians (n=1,708), Hispanic (n=1,064), and Chinese-American (n=559). Age, race/ethnicity, sex, baseline measurements including the use of medications, systolic blood pressure, diabetes (treated or untreated diabetes mellitus as determined by 2003 American Diabetes Association fasting criteria algorithm), smoking status (former and current), and education status were recorded.

Laboratory measurements

Lp(a) mass concentration was measured in baseline specimens by Health Diagnostics Laboratory (Richmond, Virginia) using a latex-enhanced turbidimetric immunoassay (Denka Seiken, Tokyo, Japan) which controls for the heterogeneous sizes of apo(a).[3] Fasting triglyceride, total cholesterol, high density lipoprotein cholesterol (HDL-C) concentrations were measured as described previously.[4] Low density lipoprotein-cholesterol (LDL-C) was calculated based on the Friedewald formula in participants with triglycerides <4.52 nmol/L. Notably, the

calculated LDL-C value includes the cholesterol component in Lp(a) particles; to account for this overlap, Lp(a)-cholesterol was also assayed by Health Diagnostics Laboratory, and values were subtracted from LDL-C to control for non-Lp(a) LDL-C. Serum phosphate levels were determined on a Beckman-Coulter UniCel DxC instrument using a timed-rate colorimetry method (interassay coefficients of variation of 2.5%).

Imaging

Coronary artery calcium (CAC) and AVC were obtained at baseline using computed tomography imaging with either electron beam or multi-detector scanners. The image acquisition protocol has been described previously.[5] Each participant was scanned twice, and the scans were interpreted at the core laboratory at the Los Angeles Biomedical Research Institute at Harbor-UCLA (University of California Los Angeles) Medical Center by experienced readers who were blinded to the clinical information. For AVC, any calcified focus that extended to the aortic root was deemed aortic valve calcium as described previously.[6]

Statistical Model

Statistical analysis was conducted using Stata (version 12.1, Stata Corp, College Station, TX). Baseline characteristics were presented as medians (interquartile range) for continuous variables and frequencies (%) for categorical variables. Missing data were excluded when calculating frequencies. The Lp(a) measures were log transformed in our analyses. Tukey-Kramer HSD was used to test differences between groups. Generalized linear model (GLM) with log link function was used to estimate the relative risk (RR) of log-Lp(a) on presence of AVC (>0 vs =0) with a 95% confidence interval (CI). AVC severity was defined as a 3-category variable: =0, 0-100, and ≥100. Ordered logistic regression was used to estimate the odds ratio of log-Lp(a) on AVC severity. The proportional odds assumption was examined using the Brant

test.[7] Statistical adjustments were made for age, sex, hypertension medication, systolic blood pressure (≥140 mm Hg), smoking status (never; former; current), existing CAC (0 or 1), serum phosphate levels, diabetes (American Diabetes Association criteria: normal glucose; impaired fasting glucose; untreated diabetes, treated diabetes), HDL-C, non-Lp(a) LDL-C and (log-transformed) triglycerides, education status (no schooling, grades 1-8; grades 9-11; completed high school; some college/no degree; technical school certificate; associate degree, bachelor's degree; graduate or professional school) and racial/ethnic group. We carried out subgroup analyses for each race/ethnicity using the same statistical model without adjustment for race. Lp(a)-race interaction was examined by including the interaction term in the regression models described above.

References

1. Bild DE, Bluemke DA, Burke GL, Detrano R, Diez Roux AV, Folsom AR, Greenland P, Jacob DR, Kronmal R, Liu K, Nelson JC, O'Leary D, Saad MF, Shea S, Szklo M, Tracy RP. Multi-ethnic study of atherosclerosis: Objectives and design. *Am J Epidemiol*. 2002;156:871-881
2. McClelland RL, Jorgensen NW, Post WS, Szklo M, Kronmal RA. Methods for estimation of disparities in medication use in an observational cohort study: Results from the multi-ethnic study of atherosclerosis. *Pharmacoepidemiol Drug Saf*. 2013;22:533-541
3. Marcovina SM, Albers JJ, Scanu AM, Kennedy H, Giaculli F, Berg K, Couderc R, Dati F, Rifai N, Sakurabayashi I, Tate JR, Steinmetz A. Use of a reference material proposed by the international federation of clinical chemistry and laboratory medicine to evaluate analytical methods for the determination of plasma lipoprotein(a). *Clin Chem*. 2000;46:1956-1967
4. Tsai MY, Johnson C, Kao WH, Sharrett AR, Arends VL, Kronmal R, Jenny NS, Jacobs DR, Arnett D, O'Leary D, Post W. Cholesteryl ester transfer protein genetic polymorphisms, hdl cholesterol, and subclinical cardiovascular disease in the multi-ethnic study of atherosclerosis. *Atherosclerosis*. 2008;200:359-367
5. Katz R, Wong ND, Kronmal R, Takasu J, Shavelle DM, Probstfield JL, Bertoni AG, Budoff MJ, O'Brien KD. Features of the metabolic syndrome and diabetes mellitus as predictors of aortic valve calcification in the multi-ethnic study of atherosclerosis. *Circulation*. 2006;113:2113-2119
6. Budoff MJ, Takasu J, Katz R, Mao S, Shavelle DM, O'Brien KD, Blumenthal RS, Carr JJ, Kronmal R. Reproducibility of ct measurements of aortic valve calcification, mitral annulus calcification, and aortic wall calcification in the multi-ethnic study of atherosclerosis. *Acad Radiol*. 2006;13:166-172
7. JS L, J F. *Regression models for categorical dependent variables using stata.* College Station, TX: Stata Press; 2014.