Published in final edited form as:

Transl Res. 2011 August ; 158(2): 92–98. doi:10.1016/j.trsl.2011.01.004.

Differential Associations of Serum Amyloid A and Pentraxin-3 with Allele-Specific Lipoprotein(a) Levels in African Americans and Caucasians

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Abstract

Lipoprotein(a) [Lp(a)] is a CVD risk factor, where inflammation impacts levels differentially across ethnicity. We investigated the effect of systemic [serum amyloid A (SAA)] and vascular [pentraxin-3 (PTX-3)] inflammation on Lp(a) levels across different apo(a) sizes in a bi-ethnic population. Lp(a) and allele-specific apo(a) levels, apo(a) sizes, SAA and PTX-3 levels were determined in 336 Caucasians and 224 African Americans. We dichotomized subjects into 2 groups using the respective median SAA (29.8 and 41.5 mg/dl for Caucasians and African Americans, respectively) or PTX-3 levels (1.6 and 1.1 ng/ml for Caucasians and African Americans, respectively). Among African Americans, but not in Caucasians, Lp(a) levels were increased (146 vs. 117 nmol/l, $P=0.024$) in the high vs. low SAA group. No difference was seen across PTX-3 groups. Further, among African Americans with smaller (<26 K4 repeats) apo(a) sizes, allele-specific apo(a) levels (111 vs. 79 nmol/l, $P=0.020$) were increased in the high vs. low SAA group. Again, no difference was seen for PTX-3. We did not find any significant associations between allele-specific apo(a) and SAA or PTX-3 levels among Caucasians with smaller (<26 K4) apo(a) sizes. In conclusion, elevated levels of SAA, but not PTX-3, were significantly associated with higher Lp(a) levels for smaller (<26 K4) apo(a) sizes in African Americans. Our results implicate that a pro-inflammatory stimulus may result in an increased cardiovascular risk through a selective increase in Lp(a) levels among African Americans carrying smaller apo(a) size.

INTRODUCTION

The role of inflammation in the pathogenesis of atherosclerotic cardiovascular disease (CVD) is well recognized, although underlying mechanisms are not completely understood. Lipoprotein(a) [Lp(a)] has been implicated as a CVD risk factor [1–4] and levels

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DISCLOSURE None.

vary across ethnicities with the most profound differences between populations of African descent, including African Americans and non Africans, e.g. Caucasians. 5-7 Lp(a) levels are to a major extent regulated by genetic factors 8 and its apolipoprotein (a) [apo(a)] component has an extensive size polymorphism due to variable number of kringle 4 (K4) repeats. 9 A large number of studies have reported an association between small size apo(a) and CVD. 10-12 Although smaller apo(a) sizes associate with higher plasma Lp(a) levels in general, there is a considerable variability in Lp(a) levels for any given apo(a) size. 13-15 Recently, the relationship between Lp(a) and inflammation has attracted attention. Lp(a) and apo(a) stimulate release of pro-inflammatory cytokines and chemokines from vascular endothelial and smooth muscle cells, as well as from monocytes and macrophages. 16-17 Furthermore, oxidized phospholipids which play an important role in atherosclerosis, are preferentially carried on Lp(a) particles in plasma. 18 Tsimikas et al and others have demonstrated that oxidized phospholipids can alter intracellular redox status and activate pro-inflammatory genes in arterial wall cells leading to inflammatory cascades in the vessel wall. 19-21

As levels of both HDL cholesterol and Lp(a) differ substantially between African Americans and Caucasians, we focused on an HDL-associated inflammatory biomarker, serum amyloid A (SAA). SAA, an acute phase reactant, is synthesized predominantly by the liver in response to pro-inflammatory cytokines such as tumor necrosis factor α (TNF α), interleukin (IL)-1 and IL-6. 22-23 Peripherally, TNF α and IL-1, but not IL-6, induce production of a long pentraxin, pentraxin-3 (PTX-3), from cells directly involved in atherosclerosis including vascular endothelial and smooth muscle cells, or monocytes and macrophages. 24-25 To assess the relationship of Lp(a) and apo(a) with inflammation in more detail, we investigated the association of allele-specific Lp(a) levels with SAA and PTX-3 in two ethnic groups (Caucasians and African Americans) with considerably different Lp(a) levels.

SUBJECTS AND METHODS

Subjects

Subjects were recruited from a patient population scheduled for diagnostic coronary angiography either at Harlem Hospital Center in New York City or at the Mary Imogene Bassett Hospital in Cooperstown, NY. The clinical characteristics of the study population and the study design including inclusion and exclusion criteria have been described previously, and notably, exclusion criteria included use of lipid lowering drugs, as well as hormone replacement therapies. 13-15-26-27 Briefly, a total of 648 patients, self-identified as Caucasian (n=344), African American (n=232), or other (n=72) were enrolled. The present report is based on the findings in 560 subjects (336 Caucasians, 224 African Americans); 16 subjects were excluded due to incomplete data. The apo(a) allele sizes, circulating apo(a) isoforms, and allele-specific apo(a) levels were available on 421 subjects (163 African Americans, 258 Caucasians). The study was approved by the Institutional Review Boards at Harlem Hospital, the Mary Imogene Bassett Hospital, Columbia University College of Physicians and Surgeons, and University of California, Davis, and informed consent was obtained from all subjects.

Measurement of plasma lipids, SAA and PTX-3 concentrations

Participants were asked to fast for 12 hours, and blood samples were drawn approximately 2 to 4 hours before the catheterization procedure. Serum and plasma samples were separated and stored at -80°C prior to analysis. Concentrations of triglycerides (Sigma Diagnostics, St. Louis, MO), total and HDL cholesterol (Roche, Somerville, NJ) were determined using standard enzymatic procedures. HDL cholesterol levels were measured after precipitation of apoB-containing lipoproteins with dextran sulfate. 28 LDL cholesterol levels were

calculated in subjects with triglyceride levels <400 mg/dl with the formula of Friedewald and apoB were measured by rate immunonephelometry (Array, Beckman, Brea, CA). Plasma Lp(a) levels were measured by an apo(a) size insensitive sandwich enzyme-linked immunosorbent assay (ELISA) (Sigma Diagnostics, St Louis, MO). 15–26 PTX-3 levels were determined by PTX3 (human) Detection Set from Alexis Biochemicals (Axxora, LLC, Plymouth Meeting, PA). 29 Plasma SAA levels were measured by a commercially available ELISA kit (Invitrogen, Inc., Carlsbad, CA) according to the manufacturer's instruction. The detection limits were 4 µg/l for SAA and 0.075 ng/ml for PTX-3.

Determination of apo(a) allele, isoform size and allele-specific apo(a) levels

To determine apo(a) allele sizes, we performed genotyping using pulsed-field gel electrophoresis of DNA from leucocytes embedded in agarose plugs. 30–31 Apo(a) isoform sizes were analyzed by SDS-agarose gel electrophoresis of plasma samples, followed by an immunoblotting. Allele-specific apo(a) levels were determined based on the computerized scanning of apo(a) protein bands on the Western blot as described previously. 26–30–31

Statistics

Analysis of data was done with SPSS statistical analysis software (SPSS Inc, Chicago, IL). Results were expressed as means $\pm$ SEM. Triglyceride, SAA and PTX-3 levels were logarithmically transformed, and Lp(a) levels and allele-specific apo(a) levels were square root transformed to achieve normal distributions. General linear measurement analyses were used for anthropometric and metabolic parameters after adjustments for age and gender. All analyses were two-tailed, and *P*-values less than 0.05 were considered statistically significant.

RESULTS

Age and gender adjusted parameters are presented in Table 1. There was no significant difference in the levels of total cholesterol, LDL cholesterol and apoB-100 between the two ethnic groups. As expected, African Americans had significantly higher levels of Lp(a) ($P<0.001$) and HDL cholesterol ($P<0.001$), and lower level of triglyceride ($P<0.001$) compared with Caucasians. Though not significantly different, SAA levels were considerably higher in African Americans compared with Caucasians (114.0 vs. 79.4 mg/l, respectively). In contrast, PTX-3 levels were significantly increased in Caucasians compared with African Americans (2.3 vs. 1.8 ng/ml, $P<0.001$) (Table 1). Since HDL cholesterol and triglyceride levels differed significantly between the two ethnic groups, we assessed the correlations of these variables with PTX-3 or SAA levels. PTX-3, but not SAA, levels were significantly and negatively associated with triglyceride levels in the both ethnic groups ($r=-0.112$, $P=0.045$ and $r=-0.150$, $P=0.026$ for Caucasians and African Americans, respectively). However, there were no significant associations between HDL cholesterol and SAA or PTX-3 in either ethnic group.

We next analyzed the effect of increased levels of SAA or PTX-3 on plasma Lp(a) levels in each ethnic group. We dichotomized study subjects into 2 groups using the respective median SAA (29.8 and 41.5 mg/dl for Caucasians and African Americans, respectively) or PTX-3 levels (1.6 and 1.1 ng/ml for Caucasians and African Americans, respectively). Among African Americans, Lp(a) levels were significantly increased (146 vs. 117 nmol/l, $P=0.024$) in the high compared to low SAA group (Figure 1, A), whereas no difference was seen across PTX-3 groups (Figure 1, B). Among Caucasians, there were no significant associations between Lp(a) and SAA or PTX-3 levels (Figure 1, A and B, respectively). Further, we studied the association of SAA or PTX-3 with allele-specific apo(a) levels. As in our previous studies, we dichotomized apo(a) sizes by using the median apo(a) size (26

K4 repeats). As seen in Figure 2, among African Americans with smaller (<26 K4) apo(a) sizes, subjects in the high SAA group had higher allele-specific apo(a) levels compared to subjects in the low SAA group (111 vs. 79 nmol/l, $P=0.020$). Again, no difference was seen for PTX-3. We did not find any significant associations between allele-specific apo(a) and SAA or PTX-3 levels among Caucasians with smaller (<26 K4) apo(a) sizes (Figure 2). Furthermore, there was no significant difference across SAA groups for African Americans carrying larger (≥ 26 K4) apo(a) sizes.

DISCUSSION

In the current study, we investigated the association of allele-specific Lp(a) levels with two less-studied inflammatory biomarkers (SAA and PTX-3) in a bi-ethnic population. Among African Americans, subjects with high SAA levels had significantly higher Lp(a) levels, whereas this association was not seen among Caucasians. Furthermore, among African Americans with smaller (<26 K4) apo(a) sizes, subjects in the high SAA group had higher allele-specific apo(a) levels compared to subjects in the low SAA group. However, we did not find any significant associations between allele-specific apo(a) and SAA levels among Caucasians with smaller (<26 K4) apo(a) sizes. Notably, PTX-3 levels were not associated with Lp(a) or allele-specific apo(a) levels in any of the ethnic group.

We previously studied the effect of inflammation on Lp(a) and allele-specific apo(a) levels across African-American-Caucasian ethnicity. Elevated levels of CRP and fibrinogen, systemic inflammatory biomarkers, were associated with higher allele-specific apo(a) levels for smaller (<26 K4 repeats) apo(a) sizes in African Americans, but not in Caucasians. 26 In a subsequent study, we demonstrated that increased levels of Lp-PLA₂, an established biomarker of vascular inflammation, were associated with higher Lp(a) levels across African-American-Caucasian ethnicity with the strongest associations observed among African Americans. 27 Importantly, Lp-PLA₂ levels were significantly associated with allele-specific apo(a) levels for smaller (<26 K4) apo(a) sizes in both African Americans and Caucasians. In contrast to the findings of the latter study, 27 PTX-3 was not associated with Lp(a) or allele-specific apo(a) levels in any of the ethnic groups. This lack of association may be due to several causes. First, although PTX-3 32, 33 and Lp-PLA₂ 34, 35 have been identified as biomarkers representing localized vascular inflammation and damage, and are synthesized by inflammatory and immune cells, there is a difference in the post-production phase. PTX-3 is produced by macrophages and dendritic cells and secreted by neutrophils, 33 partially remaining cell-associated (presumably in neutrophil extracellular traps). 36 It acts as an acute-phase response protein with a rapid (peak at 6-8h) and pronounced (>100-fold) increase during inflammatory and infectious conditions, correlating with the severity of the disease 32. On the contrary, following its secretion by leukocytes, Lp-PLA₂ in plasma binds to lipoproteins such as LDL, HDL and Lp(a), for the latter in particular when plasma levels exceed 30 mg/dl. 37 Notably, Lp(a) particles carry proportionally more Lp-PLA₂ mass (1.5-2-fold) and activity (up to 7-fold) compared to equimolar amounts of LDL 37. Furthermore, Tsimikas et al suggested that Lp(a)-associated Lp-PLA₂ may have an important pro-atherogenic role impacting on phospholipids. 38 Thus, unlike PTX-3, Lp-PLA₂ possesses a close relationship with Lp(a), which modulates its enzymatic activity. We can not rule out that these differences may contribute to the differential association pattern between these biomarkers and Lp(a). Thus, although both PTX-3 and Lp-PLA₂ are considered as vascular inflammatory biomarkers, it is likely that differences in the properties of the two biomarkers might affect their roles in promoting a vascular inflammatory pattern. Taken together these possibilities underscore the complexity of the relationships between inflammation, atherosclerosis and plasma lipids in health and disease, as well as across different ethnic populations.

Compared to the levels in our study, PTX-3 levels were higher in an elderly (≈ 70 years) multi-ethnic population (1.9 and 1.5 ng/ml for Caucasians and African Americans, respectively), and were associated with CVD and all-cause deaths. 29 Since PTX-3 expression and level is reported to increase as atherosclerotic lesions progress from fatty streak to advanced lesions, 39 further exploration of the relationship between PTX-3 and Lp(a) across the lifespan is of interest. In addition, our findings that PTX-3 levels were higher in Caucasians compared to African Americans (1.6 vs. 1.1 ng/ml) were in agreement with the results of the former study. 29

Lp(a) and apo(a) mRNA levels in primary monkey hepatocytes were responsive to pro-inflammatory cytokines, and their expressions were positively regulated by IL-6, the major mediator of an acute-phase response, and negatively regulated by TNF α and transforming growth factor- β 1. 40 Thus, *in vivo* Lp(a) levels may depend on the balance between stimulatory and inhibitory cytokines. The liver produces increased amounts of SAA in response to TNF α , IL-1 and IL-6. 22, 23 On the other hand, PTX-3 was shown to be transcribed after exposure to both TNF α and IL-1 β , but not to IL-6 in several different cell types. 24 In addition, PTX-3 expression induced by IL-1 β was not modified by concomitant exposure to IL-6 in endothelial cells and hepatocytes. 24 These variable responses of the inflammatory biomarkers to cytokines may also add an additional explanation to our finding of SAA, but not PTX-3, was associated with Lp(a) levels.

An interesting observation when taking our previous 26, 27 and the current study together is a contrasting pattern in the levels of systemic and vascular inflammatory biomarkers across African-American-Caucasian ethnicity. As summarized in Figure 3, African American subjects had higher levels of systemic and lower levels of vascular inflammatory biomarkers compared with Caucasian subjects. These findings indicate that there might be an interethnic difference with regard to changes in systemic vs. vascular inflammatory biomarkers for this high risk group of subjects. However, further studies are needed to address to what extent marker patterns might differ between various subpopulations. Regardless of this differential pattern, the associations of both types of inflammatory biomarkers with Lp(a), e.g. allele-specific apo(a) levels were stronger and more pronounced among African Americans. It is well recognized that African Americans have substantially higher Lp(a) levels compared with other ethnicities including Caucasians. 5-7 Although Lp(a) levels are to a major extent regulated by genetic factors, 8 we hypothesized that pro-inflammatory conditions may affect the Lp(a) levels, thus contributing to its interethnic difference. Overall, our findings suggest the potential for an additive effect between Lp(a), in particular Lp(a) carrying small size apo(a), and inflammation in promoting cardiovascular risk. It is in this context of interest that small size apo(a) has been implicated as a cardiovascular risk factor in many studies. 4, 7, 10-12 Again beyond underscoring an impact of inflammation on Lp(a) levels, the findings of the current study reinforce the concept that inflammation-associated events may contribute to the Lp(a) interethnic difference.

We acknowledge some of the limitations of this study. Subjects in our study were recruited from patients scheduled for coronary angiography and are likely more typical of a high-risk patient group than the healthy population at large. However, none of the patients had a history of acute coronary symptoms or surgical intervention within 6 months, arguing against any secondary increase in inflammatory parameters due to an acute CAD. Further, clinical and laboratory parameters were in agreement with differences generally observed between healthy African American and Caucasian populations from other studies. Although the subjects in our study may not be fully representative of the healthy population, our findings still suggest that even within a high-risk population, pro-inflammatory conditions may differentially influence Lp(a) levels across African-American-Caucasian ethnicity.

In conclusion, this is to our knowledge the first study to examine the relationship between apo(a) size, allele-specific apo(a) levels and SAA or PTX-3 in a bi-ethnic population. An important finding was that elevated levels of SAA were significantly associated with increased Lp(a) levels for smaller (<26 K4 repeats) apo(a) sizes in African Americans, emphasizing the importance of small size apo(a) as a cardiovascular risk factor. We did not find any significant association between PTX-3 and allele-specific apo(a) levels. Our results implicate that a systemic pro-inflammatory stimulus may result in a selective increase in Lp(a) levels among African Americans carrying smaller apo(a) size, which might contribute to an increased cardiovascular risk. Further studies are needed to verify these results in other populations and to explore whether systemic and vascular inflammation contributes to the interethnic difference in Lp(a) levels and impact on the role of Lp(a) as risk factor for CVD.

Acknowledgments

Supported by grant HL 62705 (to L.B.) from the National Heart, Lung and Blood Institute and in part by the UC Davis Clinical and Translational Research Center (RR024146). EA is a recipient of an UC Davis Clinical and Translational Science Center K12 Award (RR024144).

Abbreviations

Apo(a)	apolipoprotein(a)
CAD	coronary artery disease
CVD	cardiovascular disease
CRP	C-reactive protein
IL	interleukin
K4	kringle 4
Lp(a)	lipoprotein(a)
Lp-PLA₂	lipoprotein-associated phospholipase A ₂
PTX-3	pentraxin-3
SAA	serum amyloid A
TNFα	tumor necrosis factor α

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Figure 1.

Associations of SAA (**A**) and PTX-3 (**B**) with Lp(a) levels across ethnicity. Subjects were dichotomized into 2 groups using the respective median SAA (29.8 mg/dl for Caucasians and 41.5 mg/dl for African Americans) or PTX-3 (1.6 ng/ml for Caucasians and 1.1 ng/ml for African Americans) levels. Square root transformed Lp(a) and logarithmically transformed SAA or PTX-3 levels were used for statistical analyses. Data are expressed as means $\pm$ SE. *: $P=0.024$

Figure 2. Associations of SAA (**A**) and PTX-3 (**B**) with allele-specific apo(a) levels for smaller (<26 K4) apo(a) sizes across ethnicity. Square root transformed allele-specific apo(a) levels and logarithmically transformed SAA or PTX-3 were used for statistical analyses. Data are expressed as means $\pm$ SE. *: $P=0.020$

Figure 3.

Distribution pattern of systemic (**A**) and vascular (**B**) inflammatory biomarkers across African-American-Caucasian ethnicity. Values for Caucasians were used as a reference (100%). Actual numbers are presented in Table 1 for SAA and PTX-3, and are given in Ref. 26 for fibrinogen and CRP, and in Ref. 27 for Lp-PLA₂ activity levels. CRP, C-reactive protein; SAA, serum amyloid A; PTX-3, pentraxin-3; Lp-PLA₂, lipoprotein-associated phospholipase A₂.

TABLE 1

Characteristics of study population.

Characteristics	Caucasians (n=336)	African Americans (n=224)	P-value
Men/Women (n)	217/119	126/98	NS
BMI (kg/m ²)	29.8 ± 0.3	28.5 ± 0.4	0.011
Total cholesterol (mg/dl)	198 ± 2	197 ± 3	NS
LDL cholesterol (mg/dl)	122 ± 2	125 ± 3	NS
HDL cholesterol (mg/dl)	41 ± 1	49 ± 1	< 0.001
Triglycerides (mg/dl)	183 ± 5	117 ± 6	< 0.001
Apolipoprotein B-100 (mg/dl)	136 ± 2	133 ± 3	NS
Lipoprotein(a) (nmol/l)	59 ± 5	132 ± 6	< 0.001
SAA (mg/l)	79 ± 9	114 ± 11	NS
PTX-3 (ng/ml)	2.3 ± 0.2 [†]	1.8 ± 0.2	< 0.001

Data are means ± SEM. General linear measurement analyses were used for anthropometric and metabolic parameters after adjustments for age and gender. Values for triglycerides, SAA and PTX-3 were logarithmically transformed before analyses.

SAA, serum amyloid A; PTX-3, pentraxin-3, NS, not significant.

[†]The result is based on the data of 324 subjects.