Racial variation in lipoprotein-associated phospholipase A₂ in older adults

Keane K Lee¹,²*, Stephen P Fortmann³,⁴†, Ann Varady⁴†, Joan M Fair⁴†, Alan S Go⁵,⁶†, Thomas Quertermous⁷†, Mark A Hlatky¹† and Carlos Iribarren⁵†

Abstract

Background: Lipoprotein-associated phospholipase A₂ (Lp-PLA₂) is a predictor of cardiovascular events that has been shown to vary with race. The objective of this study was to examine factors associated with this racial variation.

Methods: We measured Lp-PLA₂ mass and activity in 714 healthy older adults with no clinical coronary heart disease and not taking dyslipidemia medication. We evaluated the association between race and Lp-PLA₂ mass and activity levels after adjustment for various covariates using multivariable linear regression. These covariates included age, sex, diabetes, hypertension, body mass index, lipid measurements, C-reactive protein, smoking status, physical activity, diet, income, and education level. We further examined genetic covariates that included three single nucleotide polymorphisms shown to be associated with Lp-PLA₂ activity levels.

Results: The mean age was 66 years. Whites had the highest Lp-PLA₂ mass and activity levels, followed by Hispanics and Asians, and then African-Americans; in age and sex adjusted analyses, these differences were significant for each non-White race as compared to Whites (p < 0.0001). For example, African-Americans were predicted to have a 55.0 ng/ml lower Lp-PLA₂ mass and 24.7 nmol/ml-min lower activity, compared with Whites, independent of age and sex (p < 0.0001). After adjustment for all covariates, race remained significantly correlated with Lp-PLA₂ mass and activity levels (p < 0.001) with African-Americans having 44.8 ng/ml lower Lp-PLA₂ mass and 17.3 nmol/ml-min lower activity compared with Whites (p < 0.0001).

Conclusion: Biological, lifestyle, demographic, and select genetic factors do not appear to explain variations in Lp-PLA₂ mass and activity levels between Whites and non-Whites, suggesting that Lp-PLA₂ mass and activity levels may need to be interpreted differently for various races.

Background

Lipoprotein-associated phospholipase A₂ (Lp-PLA₂) circulates in the blood as an enzyme bound mainly to low-density lipoprotein (LDL) particles. Lp-PLA₂ has been found in multiple studies to be associated with incident and prevalent coronary heart disease (CHD) [1-6] and incident stroke,[5,7] independent of standard cardiovascular risk factors[8]. A collaborative meta-analysis of 32 prospective studies in 79,000 patients showed that both Lp-PLA₂ mass and activity added predictive value for vascular events and mortality beyond traditional cardiovascular risk factors[9]. Currently, a direct Lp-PLA₂ inhibitor is being tested in a randomized trial for prevention of cardiovascular events in patients with CHD[10]. In a study of the general population, Lp-PLA₂ mass and activity levels were higher in Whites compared with African-Americans or Hispanics[11]. However, the degree to which genetic differences as opposed to lifestyle variations, including smoking status, diet and exercise, may explain racial differences remains unclear. The goals of this study were to confirm the racial variations in Lp-PLA₂ mass and activity levels and to examine the factors that might explain these racial differences in a multi-racial cohort of healthy older individuals free of clinical CHD and not taking dyslipidemia medications.
Methods
Study Sample
The participants in this study were recruited from Kaiser Permanente Northern California, a large integrated healthcare system that provides comprehensive care to more than 3 million people in San Francisco and the greater Bay Area. Details of participant recruitment have been described previously[12,13]. Briefly, we enrolled adults between 60 and 72 years old who lived within 50 miles of the research facility, and who did not have a diagnosis of cardiovascular disease, cancer, end-stage renal disease, liver failure, dementia, or human immunodeficiency virus infection. By January 2001, 3,054 apparently eligible subjects were randomly chosen to be sent letters inviting participation. From these invitations, a total of 1,024 subjects were recruited, consisting of 639 men and 385 women. In the final year of recruitment, men, Hispanics, and African-Americans were selectively oversampled in order to better match the cases enrolled in the Atherosclerotic Disease, Vascular Function, and Genetic Epidemiology study[14]. After excluding people who were taking dyslipidemia medications from this analysis, 714 subjects were included in the study. The study protocol was reviewed and approved annually by the institutional review boards at both Stanford University and the Division of Research at Kaiser Permanente Northern California. Written informed consent was obtained from all participants.

Risk Factor Measurement
All the study subjects attended a research clinic visit at Stanford University and completed a health survey about medical diagnoses, medication use, smoking history, family history, race and ethnicity, dietary intake, and physical activity. Subjects were asked to bring their medications, which were independently reviewed and recorded by the interviewer. Resting blood pressure was determined using standard sphygmomanometers. Weight, height, and waist circumference were measured by trained and certified staff using a stadiometer, balance scale, and tape. Blood tests were drawn for biomarker analysis, which included fasting lipid levels, glucose, C-reactive protein,[15] and Lp-PLA2 mass and activity levels[16,17].

Stanford Seven-Day Physical Activity Recall
The Stanford Seven-Day Physical Activity Recall, a semi-structured interview, was used to estimate the amount of time that a person engaged in moderate-, hard-, and very hard-intensity activities from the total hours in the recall period. Total energy expenditure was estimated by multiplying the hours spent in each level of activity with the estimated energy expenditure value for each intensity category.

Block Food Frequency Questionnaire
The Block Food Frequency Questionnaire was used to characterize dietary intake[20]. It queries average consumption of 106 foods by portion size and includes 13 questions on dietary supplementation, six questions on restaurant eating, five summary questions, eight questions on fat use or low-fat foods, and seven demographic and health-related questions.

Race Determination
Self-reported race was collected during the eligibility screening survey, on the baseline health survey and from the Kaiser Permanente data sources. In addition, birthplace, grandparents’ race, and grandparents’ country of origin were collected. The algorithm for assigning race was as follows: if self-reported race and grandparents’ race were concordant, then subjects were coded to that race category (> 80% of subjects). In discordant cases, race was assigned by a hierarchy of grandparents’ race, grandparents’ country of origin, self-reported race on the baseline health survey, and self-reported race on the screening survey. Only the major racial groups of White, African-American, Hispanic and Asian are used for this analysis. This method of classifying race has been validated in this cohort by high-throughput genotyping of more than 450,000 single nucleotide polymorphisms (SNPs) among African-Americans and Whites[21].

Lp-PLA2 Measurement
Blood samples were collected at the baseline visit after an overnight fast and stored in aliquots frozen at -80 °C. Lp-PLA2 mass (ng/ml) was measured using a dual enzyme linked immunoassay (PLAC test, diaDexus Inc., South San Francisco, CA)[16]. Intra- and inter-assay coefficients of variation were < 5% and < 8%, respectively, and sensitivity across the assay range was < 0.5 ng/mL. Lp-PLA2 activity (nmol/ml-min) was measured by a colorimetric activity method (CAM test)[17]. Intra- and inter-assay coefficients of variation were < 4% and < 6%, respectively, and sensitivity across the assay range was < 5 nmol/ml-min.

Single Nucleotide Polymorphism Selection
A literature search for all of the documented SNPs related to Lp-PLA2 mass and activity levels was conducted. Only those SNPs that were both documented in the literature to be related to Lp-PLA2 and existed in our database were included in the analysis. The SNPs included in our database were selected for likely
association with atherosclerosis as determined *a priori* from literature review and by comparing up and down-regulated genes in diseased and non-diseased vascular endothelium. The genetic covariates included three SNPs in the PLA2G7 locus (Lp-PLA2 gene) that have been documented to be associated with Lp-PLA2 activity: Ala379Val (rs1051931), Arg92His (rs1805017), and Ile198Thr (rs1805017)[22]. The SNPs were coded as 0 for homozygous major, 1 for heterozygous, and 2 for homozygous minor.

**Statistical methods**

Baseline characteristics were presented as means and standard deviations for normally distributed continuous variables and as counts and proportions for categorical variables. Medians and interquartile ranges were used for non-normally distributed continuous variables. Differences in the baseline variables between racial groups were compared using a general linear model analysis of variance (ANOVA).

Linear regression was used to examine the association between race and Lp-PLA2 mass and activity in separate models. The covariates for multivariable analysis were selected *a priori* and included biological, lifestyle, demographic, and genetic characteristics. The biological covariates included age, race, history of diabetes mellitus, history of hypertension, quantitative systolic blood pressure measurement, body mass index (BMI), waist circumference, LDL cholesterol level, high density lipoprotein (HDL) cholesterol level, asymmetric dimethylarginine (ADMA), and C-reactive protein (CRP); the lifestyle and demographic covariates were current smoking status, physical activity, percent of calories from saturated fat, percent of calories from carbohydrates, education level, and income.

A simple linear regression model was first used to analyze the relation between race and Lp-PLA2 mass and activity adjusted for only sex. The biological, lifestyle, and genetic covariates were each included in separate multivariable models in addition to a single multivariable model with all the covariates. Data were analyzed using SAS, version 9.1 (SAS Institute, Cary, North Carolina).

**Results**

The study population consisted of 540 whites, 60 African-Americans, 62 Hispanics, and 52 Asians (Table 1). The average age was 66 years and women comprised 40% of the study cohort. The prevalence of hypertension (62%) was slightly higher and the prevalence of diabetes (13%) relatively lower than the general population[23,24]. Mean LDL levels were mildly elevated (128 mg/dL), while mean HDL levels were relatively high (54 mg/dL). The cohort was overweight (mean BMI of 28 kg/m²) and only a small proportion were active smokers (7.3%). Some baseline characteristics differed significantly among the racial groups (Table 1). The study had insufficient power to compare the non-White racial groups to each other, so all statistical comparisons are to Whites. Whites were the least likely to be diabetic, while in comparison, African-Americans were the most likely to be diabetic. Asians had the lowest mean BMI and smallest mean waist circumference. Asians and Hispanics were significantly less likely to be actively smoking as compared with Whites. Whites had a lower dietary intake of saturated fat and higher intake of from carbohydrates (both as percent of total energy). LDL and HDL levels were relatively similar comparing White with non-White groups. There were also significant differences in the SNP frequencies between White and non-White races. Compared with African-Americans and Hispanics, Whites were more likely to have the Ala379Val SNP. Hispanics were the most likely to have the Arg92His SNP, while African-Americans were the most likely to have the Ile198Thr SNP compared to Whites.

Men had significantly higher Lp-PLA2 mass and activity levels than women, after adjusting for all covariates (p = 0.025). The mean Lp-PLA2 mass for men was 246 ng/ml compared with 223 ng/ml for women. The mean Lp-PLA2 activity for men was 142 nmol/ml-min compared with 114 nmol/ml-min for women. There was no evidence of an interaction between sex and racial variations in Lp-PLA2 mass or activity in any model, so analyses were conducted in the combined cohort of men and women. A secondary analysis, which included subjects taking dyslipidemia medications, showed the same overall findings and confirmed that excluding these patients did not significantly change the final results.

Lp-PLA2 mass and activity levels varied significantly between Whites and non-Whites in all linear regression models (Figures 1 and 2 and Tables 2 and 3). Whites had the highest Lp-PLA2 mass and activity levels, followed by Hispanics. African-Americans and Asians had the lowest Lp-PLA2 mass and activity levels. These differences between Whites and non-Whites remained highly significant even after adjusting for all covariates. In fact, the values of the β coefficients for non-White races were high, ranging from -25 to -55 ng/ml across models for predicting Lp-PLA2 mass and -10 to -28 nmol/ml-min for predicting Lp-PLA2 activity. The other covariates with strong associations with Lp-PLA2 mass and activity levels included age, LDL, HDL, and ADMA levels. The Arg92His SNP in the PLA2G7 locus was associated with lower Lp-PLA2 activity, but higher Lp-PLA2 mass, whereas the other two SNPs had no significant associations.

None of the other covariates were consistently associated with Lp-PLA2 mass and activity levels. Hypertension and diabetes were weakly associated with Lp-PLA2 activity and not associated with mass. Conversely,
C-reactive protein was weakly associated with Lp-PLA\(_2\) mass, but not activity. None of the lifestyle covariates were significantly associated with Lp-PLA\(_2\) mass or activity levels.

**Discussion**

Our study confirms that Lp-PLA\(_2\) mass and activity levels vary significantly between White and non-White races in healthy older adults without clinically diagnosed CHD and not taking dyslipidemia medications. Whites have the highest Lp-PLA\(_2\) mass and activity levels, Hispanics have intermediate levels, and African-Americans and Asians have the lowest Lp-PLA\(_2\) mass and activity levels. These relationships were not affected by statistical adjustment for lifestyle and demographic factors, standard biological risk factors, and three SNPs known to be associated with Lp-PLA\(_2\) activity levels. These findings suggest that differences in Lp-PLA\(_2\) mass and activity levels between Whites and non-Whites may be due to other genetic factors or perhaps to unmeasured lifestyle factors.

The differences between White, Hispanic, and African-American race and Lp-PLA\(_2\) mass and activity levels in our study were similar to the results from the Dallas Heart Study, the largest community-based study of Lp-PLA\(_2\) levels[11]. One key difference was that our

### Table 1 Baseline characteristics among older adults without clinical cardiovascular disease by major race subgroups; mean (s.d.) and N (%)

| Variable                                | All subjects (n = 714) | White (n = 540) | African-American (n = 60) | Hispanic (n = 62) | Asian (n = 52) | P-value* |
|-----------------------------------------|------------------------|-----------------|---------------------------|------------------|---------------|----------|
| Age (years)                             | 65.8 (2.9)             | 65.7 (2.9)      | 65.5 (3.0)                | 66.3 (2.8)       | 65.9 (2.5)    | 0.38     |
| Female gender                           | 284 (40%)              | 216 (40%)       | 58 (11%)                  | 15 (25%)         | 10 (16%)      | 0.011    |
| Diabetes mellitus                       | 91 (13%)               | 58 (11%)        | 15 (25%)                  | 10 (16%)         | 39 (63%)      | 0.095    |
| History of hypertension                 | 440 (62%)              | 321 (59%)       | 45 (75%)                  | 39 (63%)         | 35 (67%)      | 0.12     |
| Systolic blood pressure (mmHg)          | 131 (18)               | 130 (17)        | 136 (22)                  | 133 (15)         | 136 (20)      | 0.0045   |
| Body mass index (kg/m\(^2\))            | 27.9 (5.0)             | 27.8 (5.0)      | 30.3 (5.8)                | 28.9 (3.9)       | 25.4 (3.6)    | < 0.0001 |
| Waist circumference (cm)                 | 93.0 (14.6)            | 92.9 (14.4)     | 98.8 (16.9)               | 95.2 (12.9)      | 84.4 (11.4)   | < 0.0001 |
| Low density lipoprotein (mg/dL)         | 128 (32)               | 128 (30)        | 125 (38)                  | 125 (32)         | 128 (36)      | 0.78     |
| High density lipoprotein (mg/dL)        | 54 (16)                | 55 (17)         | 56 (17)                   | 53 (15)          | 53 (13)       | 0.61     |
| Triglycerides (mg/dL)\(^†\)             | 116 (86)               | 118 (84)        | 97 (64)                   | 138 (113)        | 121 (89)      | 0.12     |
| C-reactive protein (mg/L)               | 0.63 (0.10)            | 0.64 (0.11)     | 0.61 (0.10)               | 0.64 (0.09)      | 0.62 (0.09)   | 0.20     |
| LpPLA\(_2\) mass (ng/mL)\(^†\)          | 227 (71)               | 233 (75)        | 198 (63)                  | 221 (59)         | 202 (73)      | < 0.0001 |
| LpPLA\(_2\) activity (nmol/ml-min)\(^†\) | 130 (43)               | 134 (41)        | 108 (32)                  | 124 (36)         | 124 (61)      | < 0.0001 |
| LpPLA\(_2\) mass > 235 ng/mL            | 302 (42%)              | 260 (48%)       | 11 (18%)                  | 19 (31%)         | 12 (24%)      | < 0.0001 |
| Current cigarette smoking               | 52 (7.3%)              | 42 (7.8%)       | 8 (13.3%)                 | 1 (16%)          | 1 (19%)       | 0.035    |
| Physical activity (kCal/kg/day)          | 34.8 (3.5)             | 34.9 (3.6)      | 34.4 (3.7)                | 35.0 (3.1)       | 34.6 (2.8)    | 0.76     |
| Percent of calories from saturated fat   | 10.1 (2.7)             | 10.3 (2.7)      | 10.3 (2.9)                | 10.1 (2.5)       | 8.5 (1.7)     | 0.0001   |
| Percent of calories from carbohydrates   | 46.6 (9.1)             | 46.0 (9.0)      | 46.3 (9.1)                | 49.1 (9.7)       | 49.8 (8.2)    | 0.0038   |
| Annual income ≥ $50,000                 | 452 (68%)              | 351 (70%)       | 37 (70%)                  | 30 (54%)         | 34 (68%)      | 0.11     |
| College graduate                        | 173 (17%)              | 133 (25%)       | 10 (17%)                  | 9 (15%)          | 21 (40%)      | 0.0059   |
| Ala379Val                               | 440 (63%)              | 340 (64%)       | 29 (40%)                  | 32 (52%)         | 39 (75%)      | 0.011    |
| Arg92His                                | 235 (33%)              | 170 (32%)       | 28 (47%)                  | 25 (41%)         | 12 (23%)      | 0.021    |
| Ile198Thr                               | 28 (4%)                | 21 (4%)         | 2 (3%)                    | 4 (7%)           | 1 (2%)        | 0.64     |
| Homozygous major                        | 394 (56%)              | 291 (55%)       | 39 (60%)                  | 37 (71%)         | 37 (71%)      | 0.011    |
| Heterozygous                            | 256 (36%)              | 198 (37%)       | 17 (29%)                  | 28 (46%)         | 13 (25%)      | 0.075    |
| Homozygous minor                        | 55 (8%)                | 44 (8%)         | 3 (5%)                    | 6 (10%)          | 2 (4%)        | 0.52     |
| Arg92His                                | 617 (87%)              | 479 (90%)       | 36 (61%)                  | 59 (97%)         | 43 (83%)      | < 0.0001 |
| Ile198Thr                               | 85 (12%)               | 52 (10%)        | 22 (37%)                  | 2 (3%)           | 9 (17%)       | < 0.0001 |

* P-value indicates the comparison across racial groups by ANOVA
† Median (interquartile range), ADMA, asymmetric dimethylarginine

http://www.biomedcentral.com/1471-2261/11/38
study also included Asians, who were found to have Lp-PLA₂ mass and activity levels that were similar to African-Americans, but lower than Whites. Our study also demonstrated that racial differences in Lp-PLA₂ mass and activity levels apply to older adults, given the fact that individuals in our study (mean age of 66 years) were substantially older than those in the Dallas Heart Study (mean age of 45 years). The Dallas Heart Study cohort included individuals taking statin medication, whereas our study excluded individuals taking any dyslipidemia medication, because such medications may reduce Lp-PLA₂ mass and activity levels[25]. Other studies that were in a small cohort[26] and in a selected population with vascular disease[27] also showed that Whites have higher Lp-PLA₂ mass and activity levels as compared with other races.

Because Lp-PLA₂ circulates bound to LDL particles, Lp-PLA₂ mass and activity levels depend greatly upon lipid metabolism and are strongly associated with LDL and HDL levels[16]. Although we also found this association, adjustment for LDL and HDL levels did not alter the relation between race and Lp-PLA₂ mass and activity levels, suggesting that variations in LDL and HDL levels do not explain these racial differences. Diet and physical activity also affect lipid metabolism,[28] but adjustment for these lifestyle factors did not attenuate the association between race and Lp-PLA₂ mass and activity levels.

Various studies have shown associations between Lp-PLA₂ mass and activity levels and different genotypes of Lp-PLA₂, bolstering the hypothesis that there is a substantial genetic influence on Lp-PLA₂ levels. A study of the Framingham cohort, using 1943 SNPs, suggested that Lp-PLA₂ mass had a multivariable adjusted heritability of 25% and Lp-PLA₂ activity had 41% heritability[29]. Among Whites, the Ala379Val coding variant in the PLA2G7 locus was associated with increased Lp-PLA₂ activity, while the Arg92His coding variant was associated with decreased activity[22]. Among Japanese individuals, the Val279Phe SNP also in the PLA2G7 locus, which is much more prevalent in Japanese than in Whites, is associated with greatly decreased Lp-PLA₂ activity[30]. In fact, people who are homozygous for the Val279Phe polymorphism have almost no detectable Lp-PLA₂ activity[31,32]. Because of the probable strong genetic influence on Lp-PLA₂ mass and activity levels, one could hypothesize that the racial variations are due to genetic differences between races. Our study included only three SNPs, while the Framingham study revealed 12 SNPs linked with Lp-PLA₂ variability[29]. Therefore, adjusting for only three SNPs in our study was a limited test of genetic influence on the racial differences in Lp-PLA₂ and did not attenuate the strong association between race and Lp-PLA₂ mass and activity levels.

In addition to the limited number of Lp-PLA₂-related SNPs, another limitation of this analysis is that it is cross-sectional as we were unable to study whether Lp-PLA₂ level predicts subsequent risk of CHD events differentially by race. Before Lp-PLA₂ results can be added to existing CHD risk prediction equations, further evidence is needed to validate its incremental prognostic value in an ethnically-diverse cohort. Another limitation is the ability of questionnaires to accurately and fully
characterize all the lifestyle variations that could explain the racial differences of Lp-PLA2 mass and activity levels. For example, there could be other lifestyle factors, such as diet choices unrelated to fat and carbohydrate intake, which influence Lp-PLA2 mass and activity levels but are not captured by the survey. The relatively modest number of non-White participants may also limit our ability to detect the effects of lifestyle factors.

Lp-PLA2 is viewed as a novel CHD risk marker that could be used to risk stratify individuals when considering primary prevention strategies, such as initiating statin therapy or setting lipid treatment goals. Various authors have suggested using a uniform threshold for Lp-PLA2 mass, regardless of sex and race, for categorizing risk. However, the results from this study confirm that Lp-PLA2 mass and activity levels vary significantly between Whites and non-Whites, independent of known biological and lifestyle factors. These racial variations could affect the performance of Lp-PLA2 as a prognostic risk marker. For example, African-Americans may have lower Lp-PLA2 mass and activity levels than Whites despite having similar degrees of atherosclerosis and vascular inflammation or they may simply have less underlying atherosclerotic disease. In light of the high cardiovascular event rates in African-Americans, the former possibility seems more likely. As a result, using the same Lp-PLA2 threshold in Whites and African-Americans may underestimate risk in African-Americans compared with Whites.

Conclusions

If Lp-PLA2 mass and activity levels are inherently different between races, then these levels may need to be interpreted differently for various races. Before incorporating information on Lp-PLA2 mass and activity levels in assessing cardiovascular risk for individual patients, additional larger, multi-racial prospective studies are needed.

Table 2 Linear regression models predicting Lp-PLA2 mass (ng/mL)

| Variables                        | Race & Sex (Model 1) | Model 1 + Biological Covariates | Model 1 + Lifestyle Covariates | Model 1 + SNPs* | All Covariates |
|----------------------------------|----------------------|---------------------------------|---------------------------------|----------------|---------------|
|                                  | b                    | P-value                         | b                              | P-value        | b             | P-value        |
| Race†                           |                      |                                 |                                 |                |               |                |
| African-American                 | -55.0                | < 0.0001                        | -50.2                           | < 0.0001       | -57.2         | < 0.0001       | -49.6         | < 0.0001       | -47.3         | < 0.0001       |
| Hispanic                         | -26.2                | 0.0016                          | -25.8                           | 0.0008         | -30.1         | 0.001          | -27.7         | 0.0008         | -30.0         | 0.0004         |
| Asian                            | -47.1                | < 0.0001                        | -45.7                           | < 0.0001       | -45.4         | < 0.0001       | -43.1         | < 0.0001       | -42.0         | < 0.0001       |
| Female gender‡                   | -24.2                | < 0.0001                        | -18.5                           | 0.0045         | -24.5         | < 0.0001       | -22.8         | < 0.0001       | -18.4         | 0.011          |
| Biological Covariates            |                      |                                 |                                 |                |               |                |
| Age (years)                      | 2.4                  | 0.0016                          | 2.6                             | 0.019          |               |                |
| Diabetes mellitus                | -0.0042              | 0.95                            | -0.018                          | 0.81           |               |                |
| History of hypertension          | -3.0                 | 0.53                            | -3.76                           | 0.49           |               |                |
| Systolic blood pressure (mmHg)   | 0.16                 | 0.23                            | 0.14                            | 0.33           |               |                |
| Body mass index (kg/m2)          | -0.46                | 0.63                            | -0.063                          | 0.95           |               |                |
| Waist circumference (cm)         | 0.092                | 0.81                            | -0.026                          | 0.95           |               |                |
| LDL (mg/dL)                      | 0.75                 | < 0.0001                        | 0.75                            | < 0.0001       |               |                |
| HDL (mg/dL)                      | -0.26                | 0.094                           | -0.27                           | 0.11           |               |                |
| ADMA§ (μmol/L)                   | 38.8                 | 0.06                            | 33.2                            | 0.14           |               |                |
| C-reactive protein (mg/L)        | 1.1                  | 0.0017                          | 1.1                             | 0.002          |               |                |
| Lifestyle/Demographic Covariates |                      |                                 |                                 |                |               |                |
| Current smoking                  | 0.012                | 0.90                            | 0.0058                          | 0.95           |               |                |
| Exercise (kCal/kg-day)           | 0.63                 | 0.40                            | 0.76                            | 0.28           |               |                |
| % Calories from saturated fat    | 0.081                | 0.94                            | -0.96                           | 0.38           |               |                |
| % Calories from carbohydrates    | -0.43                | 0.20                            | -0.60                           | 0.058          |               |                |
| Annual income ≥ $50,000          | -0.019               | 0.74                            | 0.051                           | 0.32           |               |                |
| College graduate                 | -0.091               | 0.086                           | -0.071                          | 0.15           |               |                |
| SNPs                             |                      |                                 |                                 |                |               |                |
| Ala379Val                        | 2.2                  | 0.60                            | 2.5                             | 0.55           |               |                |
| Arg92His                         | 11.8                 | 0.0023                          | 11.6                            | 0.0025         |               |                |
| Ile198Thr                        | -8.8                 | 0.19                            | -6.1                            | 0.37           |               |                |

* SNPs, single nucleotide polymorphisms  
† Compared to White race  
‡ Compared to Male  
§ADMA, asymmetric dimethylarginine
needed to delineate the specific risk associated with different Lp-PLA2 levels within individual racial groups.

Acknowledgements
We thank Themistocles L. Assimes, MD, PhD for statistical advice.

Author details
1Department of Health Research and Policy, Stanford University School of Medicine, HRP Redwood Building, Stanford, CA 94305-5405 USA. 2Kaiser Santa Clara Cardiology, Department 348, Kaiser Permanente of Northern California, 710 Lawrence Expressway, Santa Clara, CA 95051 USA. 3Center for Health Research, Kaiser Permanente Northwest, 3800 N. Interstate Avenue, Portland, OR 97227 USA. 4Stanford Prevention Research Center, Stanford University School of Medicine, 300 Pasteur Drive, Falk Cardiovascular Research Center, Stanford, CA 94305-5406 USA. 5Division of Research, Kaiser Permanente of Northern California, 2000 Broadway, Oakland, CA 94612 USA. 6Department of Epidemiology, Biostatistics, and Medicine, University of California, San Francisco, 185 Berry Street, Lobby 5, Suite 5700, San Francisco, CA 94158-0107 USA. 7Cardiovascular Medicine, Stanford University School of Medicine, 300 Pasteur Drive, Falk Cardiovascular Research Center, Stanford, CA 94305-5406 USA.

Table 3 Linear regression models predicting Lp-PLA2 activity (nmol/mL-min)

| Variables                        | Race & Sex (Model1) | Model1 + Biological Covariates | Model 1 + Lifestyle Covariates | Model1 + SNPs* | All Covariates |
|----------------------------------|---------------------|--------------------------------|-------------------------------|----------------|----------------|
|                                  | β                   | P-value                         | β                             | P-value        | β                           | P-value |
| Race                             |                     |                                 |                               |                |                             |         |
| African-American                 | -24.7               | < 0.0001                        | -18.5                         | < 0.0001       | -27.0                       | < 0.0001 | -22.8                       | < 0.0001 | -18.0                       | < 0.0001 |
| Hispanic                         | -10.1               | 0.009                           | -10.6                         | 0.0006         | -14.4                       | 0.0006  | -10.2                       | 0.009    | -13.1                       | 0.001    |
| Asian                            | -12.1               | 0.003                           | -14.2                         | < 0.0001       | -10.4                       | 0.018   | -11.9                       | 0.004    | -13.9                       | 0.0001   |
| Female gender                    | -28.1               | < 0.0001                        | -18.2                         | < 0.0001       | -28.3                       | < 0.0001 | -27.6                       | < 0.0001 | -17.4                       | < 0.0001 |
| Biological Covariates            |                     |                                 |                               |                |                             |         |
| Age (years)                      | 1.05                | 0.0006                          | 1.13                          | 0.0006         |                             |         |
| Diabetes mellitus                | -0.057              | 0.038                           | -0.067                        | 0.024          |                             |         |
| History of hypertension          | -2.38               | 0.23                            | -3.65                         | 0.094          |                             |         |
| Systolic blood pressure (mmHg)   | -0.0034             | 0.95                            | 0.0012                        | 0.98           |                             |         |
| Body mass index (kg/m2)          | -0.41               | 0.29                            | -0.34                         | 0.39           |                             |         |
| Waist circumference (cm)         | 0.041               | 0.79                            | -0.0066                       | 0.97           |                             |         |
| LDL (mg/dL)                      | 0.44                | < 0.0001                        | 0.45                          | < 0.0001       |                             |         |
| HDL (mg/dL)                      | -0.69               | < 0.0001                        | -0.72                         | < 0.0001       |                             |         |
| ADMA§ (μmol/L)                   | 23.4                | 0.0048                          | 23.1                          | 0.012          |                             |         |
| C-reactive protein (mg/L)        | -0.23               | 0.10                            | -0.22                         | 0.13           |                             |         |
| Lifestyle/Demographic Covariates |                     |                                 |                               |                |                             |         |
| Current smoking                  | 0.035               | 0.43                            | 0.025                         | 0.49           |                             |         |
| Exercise (kCal/kg-day)           | 0.36                | 0.30                            | 0.38                          | 0.17           |                             |         |
| % Calories from saturated fat    | 1.09                | 0.038                           | 0.047                         | 0.91           |                             |         |
| % Calories from carbohydrates    | 0.31                | 0.045                           | -0.055                        | 0.66           |                             |         |
| Annual income ≥ $50,000          | -0.023              | 0.38                            | 0.011                         | 0.60           |                             |         |
| College graduate                 | -0.0095             | 0.70                            | 0.014                         | 0.48           |                             |         |
| SNPs                             |                     |                                 |                               |                |                             |         |
| Ala379Val                        | 1.20                | 0.55                            | 1.45                          | 0.40           |                             |         |
| Arg92His                         | -2.06               | 0.26                            | -3.75                         | 0.015          |                             |         |
| ile198Thr                        | -6.03               | 0.056                           | -4.38                         | 0.11           |                             |         |

* SNPs, single nucleotide polymorphisms
† Compared to White race
‡ Compared to Male
§ ADMA, asymmetric dimethylarginine

Authors’ contributions
SPF, JMF, CI, ASG, MAH, and TQ conceived of the study. AV, KKL, JMF, SPF, and CI had input to the statistical analyses. KKL drafted the manuscript. All authors edited the manuscript, approved the analyses, and read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Received: 28 May 2010 Accepted: 29 June 2011 Published: 29 June 2011

References
1. Corson MA, Jones PH, Davidson MH: Review of the evidence for the clinical utility of lipoprotein-associated phospholipase A2 as a cardiovascular risk marker. Am J Cardiol 2008, 101:41F-50F.
2. Garza CA, Montori VM, McConnell JP, Somers VK, Kullo IJ, Lopez-Jimenez F: Association between lipoprotein-associated phospholipase A2 and cardiovascular disease: a systematic review. Mayo Clin Proc 2007, 82:159-65.
3. Ballantyne CM, Hoogeveen RC, Bang H, Coresh J, Folsom AR, Heiss G, Sharrett AR: Lipoprotein-associated phospholipase A2, high-sensitivity C-reactive protein, and risk for incident coronary heart disease in middle-
aged men and women in the Atherosclerosis Risk in Communities (ARIC) study. **Circulation** 2004, 109:837-42.

4. Koeng W, Khusseyonova N, Lowell H, Trischler G, Meisinger C. Lipoprotein-associated phospholipase A2 adds to risk prediction of incident coronary events by C-reactive protein in apparently healthy middle-aged men from the general population: results from the 14-year follow-up of a large cohort from southern Germany. **Circulation** 2004, 110:1903-8.

5. Oei HH, van der Meer IM, Hofman A, Koudstaal PJ, Stijnen T, Breteler MM, Wittmer JC. Lipoprotein-associated phospholipase A2 activity is associated with risk of coronary heart disease and ischemic stroke: the Rotterdam Study. **Circulation** 2005, 111:570-5.

6. Packard CJ, O'Neil LS, Caslake MJ, McMahon AD, Ford I, Cooney J, Macphee CH, Suckling KE, Krishna M, Wilkinson FE, Rumley A, Lowe GD. Lipoprotein-associated phospholipase A2 as an independent predictor of coronary heart disease. West of Scotland Coronary Preventive Study Group. **N Engl J Med** 2000, 343:1148-55.

7. Ballantyne CM, Hoogeveen RC, Bang H, Coresh J, Folsom AR, Chambless LE, Myerson M, Wu KK, Sharrett AR, Boerwinkle E. Lipoprotein-associated phospholipase A2, high-sensitivity C-reactive protein, and risk for incident ischemic stroke in middle-aged men and women in the Atherosclerosis Risk in Communities (ARIC) study. **Arch Intern Med 2005**, 165:2479-85.

8. Iribarren C. Lipoprotein-Associated Phospholipase A2 and C-Reactive Protein for Measurement of Inflammatory Risk: Independent or Complementary? **Cardiovascular Risk Reports** 2010.

9. Thompson A, Gao P, Orle L, Watson S, Di Angelantonio E, Kaptoge S, Ballantyne C, Cannon CP, Criqui M, Cushman M, Hoffman A, Packard C, Thompson SG, Collins R, Danesh J. Lipoprotein-associated phospholipase A2 and risk of coronary disease, stroke, and mortality: collaborative analysis of 32 prospective studies. **Lancet** 2010, 375:1536-44.

10. White H, Held C, Stewart R, Watson D, Harrington R, Budaj A, Steg PG, Cannon CP, Krug-Gourley S, Wittes J, Trivedi T, Tarka E, Wallentin L. Influence of race and sex on lipoprotein-associated phospholipase A2 activity in patients with coronary heart disease. **Circulation** 2009, 119:749-55.

11. Brilakis ES, Khera A, McGuire DK, See R, Banerjee S, Murphy SA, de Lemos JA. Racial differences in coronary artery calcium in a healthy cohort aged 60 to 69 years. **Atherosclerosis** 2008, 199:110-5.

12. Fair JM, Klaz mond A, Varady A, Mahbouba M, Norton L, Rubin GD, Iribarren C, Go AS, Hlatky MA, Fortmann SP. Ethnic differences in coronary artery calcium in a healthy cohort aged 60 to 69 years. **Am J Cardiol 2007**, 100:981-5.

13. Taylor-Piliae RE, Norton LC, Haskell WL, Mahbouba NH, Fair JM, Iribarren C, Hlatky MA, Go AS, Fortmann SP. Validation of a new brief physical activity survey among men and women aged 60-69 years. **Am J Epidemiol 2006**, 164:598-606.

14. Assimes TL, Koeng W, Trisc hler G, Meisinger C. Lipoprotein-associated phospholipase A2 levels: observations from the Dallas Heart Study. **Atherosclerosis** 2008, 199:110-5.

15. Ballantyne CM, Hoogeveen RC, Bang H, Coresh J, Folsom AR, Chambless LE, Myerson M, Wu KK, Sharrett AR, Boerwinkle E. Lipoprotein-associated phospholipase A2, high-sensitivity C-reactive protein, and risk for incident ischemic stroke in middle-aged men and women in the Atherosclerosis Risk in Communities (ARIC) study. **Arch Intern Med 2005**, 165:2479-85.

16. White H, Held C, Stewart R, Watson D, Harrington R, Budaj A, Steg PG, Cannon CP, Krug-Gourley S, Wittes J, Tarka E, Tarka T, Tarka A, Wallentin L. Influence of race and sex on lipoprotein-associated phospholipase A2 activity in patients with coronary heart disease. **Circulation** 2009, 119:749-55.

17. Brilakis ES, Khera A, McGuire DK, See R, Banerjee S, Murphy SA, de Lemos JA. Racial differences in coronary artery calcium in a healthy cohort aged 60 to 69 years. **Am J Cardiol 2007**, 100:981-5.

18. Taylor-Piliae RE, Norton LC, Haskell WL, Mahbouba NH, Fair JM, Iribarren C, Hlatky MA, Go AS, Fortmann SP. Validation of a new brief physical activity survey among men and women aged 60-69 years. **Am J Epidemiol 2006**, 164:598-606.

19. Brilakis ES, Khera A, McGuire DK, See R, Banerjee S, Murphy SA, de Lemos JA. Racial differences in coronary artery calcium in a healthy cohort aged 60 to 69 years. **Am J Cardiol 2007**, 100:981-5.

20. Brilakis ES, Khera A, McGuire DK, See R, Banerjee S, Murphy SA, de Lemos JA. Racial differences in coronary artery calcium in a healthy cohort aged 60 to 69 years. **Am J Cardiol 2007**, 100:981-5.

21. Zakhara F, Basu A, Absher D, Assimes TL, Knowles JW, Priest JR, Basu A, Borchert A, Volcik KA, Grove ML, Assimes TL, Knowles JW, Priest JR, Basu A, Borchert A, Volcik KA, Grove ML. Lipoprotein-associated phospholipase A2 as an independent predictor of coronary heart disease independently of traditional risk factors, markers of inflammation, renal function, and hemodynamic stress. **Atheroscler Thromb Vasc Biol 2006**, 26:1586-93.

22. Blair SN, Haskell WL, Hoogeveen RC, Bang H, Coresh J, Folsom AR, Chambless LE, Myerson M, Wu KK, Sharrett AR, Boerwinkle E. Lipoprotein-associated phospholipase A2, high-sensitivity C-reactive protein, and risk for incident ischemic stroke in middle-aged men and women in the Atherosclerosis Risk in Communities (ARIC) study. **Arch Intern Med 2005**, 165:2479-85.