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Antiphospholipid antibodies and sub-clinical atherosclerosis in the Coronary Artery Risk Development in Young Adults (CARDIA) cohort

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Abstract

Objective and design—Antiphospholipid antibodies (APA) have been associated with clinical cardiovascular disease, but it remains unclear whether APA are associated with sub-clinical atherosclerosis. This study examined the relationship between APA and sub-clinical atherosclerosis, measured as coronary artery calcification (CAC), in participants from the prospective Coronary Artery Risk Development in Young Adults (CARDIA) Study.

Subjects and method—2,203 black and white participants with sera available from the CARDIA year 7 examination and CAC measured by computed tomography at years 15 or 20 were selected.

Results—Anti- β 2-glycoprotein I (anti- β 2-GPI) immunoglobulin (Ig) M, IgG, and IgA were positive in 7.0, 1.4, and 1.8 % of participants, respectively; anti-cardiolipin (aCL) IgM and IgG were positive in 1.5 and 1.0 %, respectively. 9.5 % of participants had CAC score >0 at year 15. Anti- β 2-GPI IgM, IgG, IgA, and aCL IgG positivity were associated with CAC >0 at year 15 after adjustment for traditional cardiovascular risk factors; [odds ratios (95 % confidence intervals) were 1.7 (1.0, 3.1), 6.4 (2.4, 16.8), 5.6 (2.3, 13.2), and 5.1 (1.4, 18.6), respectively]. Anti- β 2-GPI IgG was associated with year 20 CAC >0, and anti- β 2-GPI IgA and aCL IgG were marginally associated.

Conclusions—These findings indicate that APA positivity during young adulthood is a risk factor for subsequent sub-clinical atherosclerosis and might play a role in the pathogenesis of atherosclerosis.

Keywords

Antiphospholipid antibodies; Cardiovascular disease; Coronary atherosclerosis; Coronary artery calcification; EBCT; MDCT

Introduction

Because risk for coronary heart disease cannot be explained completely by the presence of traditional cardiac risk factors, new approaches to cardiovascular risk assessment and identification of novel biomarkers for early coronary heart disease are being sought in order to identify more patients who are asymptomatic but at sufficiently high risk to justify risk factor modification [1]. Autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus have an increased prevalence of atherosclerosis [2, 3]. Moreover, there are preliminary data showing that circulating autoantibodies are associated with coronary heart disease [4-7]. These observations have led to hypotheses that coronary heart disease has autoimmune features [8].

In particular, antiphospholipid antibodies (APA) have been found in the sera and plaques of individuals with atherosclerosis and clinical cardiovascular events [5, 6, 8, 9]. It has been hypothesized that they contribute to atherosclerosis through both thrombotic and inflammatory pathways [6]. For example, evidence for the proatherogenic property of APA has been demonstrated in mouse models of antiphospholipid syndrome and mouse models of atherosclerosis [10-14]. Moreover, if APA are associated with subclinical atherosclerosis in humans prior to the onset of clinical thrombotic events, then it is possible that their association with atherosclerosis is due to non-thrombotic pathways. A few studies demonstrated a correlation between APA [15, 16] or antiphospholipid syndrome [17] and intima media thickness. However, the results were inconsistent and most associations were found in individuals with active autoimmune connective tissue diseases or primary antiphospholipid syndrome. Previously, there was no known association between APA and coronary artery calcification (CAC), a marker of overall atherosclerotic burden [5, 18, 19].

However, in a small group of lupus patients ($N = 60$), Plazak et al. [20] recently demonstrated an increased risk of CAC in patients with elevated anticardiolipin (aCL) and anti- β 2-glycoprotein I (anti- β 2-GPI) immunoglobulin (Ig) G levels. In this study, we tested the hypothesis that circulating APA are associated with subsequent sub-clinical atherosclerosis measured as CAC in a cohort of community-based young adults.

Materials and methods

Study population

The study population was drawn from the Coronary Artery Risk Development in Young Adults (CARDIA) cohort which was initiated in 1985 to describe the distribution of risk factors for coronary heart disease. CARDIA recruited 5,115 healthy participants aged 18–30 years and roughly evenly balanced by gender, black and white race, and education. CARDIA participants were examined at 2–5-year intervals at four centers, Chicago, Minneapolis, Birmingham, and Oakland. The overall design of the CARDIA study has been previously described [21]. All CARDIA examinations were approved by institutional review boards at each site, and informed consent was obtained from each study participant. This ancillary study to CARDIA also received institutional review board approval. For this ancillary study, participants with stored serum from the CARDIA year 7 examination in 1992 as well as data for CAC at CARDIA years 15 and/or 20 were selected (Fig. 1). We excluded participants who were pregnant at the year 7 examination and participants missing data for pertinent covariates. We examined the relationship between APA and CAC in 1,838 participants with data for CAC at the CARDIA year 15 examination and in 1,880 participants with data for CAC at the year 20 examination (Fig. 1).

Antiphospholipid antibody measurement

Anti- β 2-GPI and aCL autoantibodies were quantified by ELISA utilizing commercial kits (TheraTest Labs Inc, Lombard, IL, USA) as previously described [22]. Each specimen was tested in two wells: one coated with antigen and one blank well without antigen. Each blank well was subtracted from the antigen-coated well to account for nonspecific binding. Standards from Louisville IgA phospholipid units (APL) Diagnostics (Seabrook, TX, USA) were used to generate the test units. The tests were performed in stages with a screening test followed by specific isotype testing in the positive screens. Although this study did not examine thrombotic outcomes, to be conservative the cutpoints used for aCL were >40 IgG phospholipid units (GPL), IgM phospholipid units (MPL) or APL based on the cutpoints suggested in the revised Sopporo criteria [23]. The cutpoints for anti- β 2-GPI were based upon inspection of the results of testing in 100 blood bank donors in order to identify outliers. They were set as follows: anti- β 2-GPI IgM >4 U/mL (96th–99th percentile), anti- β 2-GPI IgG >25 U/mL (98th–99th percentile), and anti- β 2-GPI IgA >4 U/mL (98th percentile). The prevalence of positive aCL IgA antibodies was zero (Table 2), so their association with CAC could not be assessed.

Quality control measures

In order to provide quality assurance, a subset of repeat paired specimens from 10 % of the study population were retested blindly. Intraclass correlation coefficients for the aCL and

anti- β 2-GPI paired results were greater than 95 %. The linear regression models for the paired results showed a nearly perfect fit, with R^2 values ranging from 0.92 to 0.98.

CAC and covariates

CARDIA measured CAC by electron-beam computed tomography (EBCT; Imatron C-150) or by four-slice multi-detector computed tomography (MDCT-4) (GE Lightspeed and Siemens Volume Zoom) at the year 15 and 20 examinations. CAC score was computed by summing Agatston score across all calcified lesions in all arteries. CAC >0 was used as the cutpoint for CAC positivity, given the lower prevalence of CAC in the young CARDIA participants (Fig. 2). CAC >0 has been used as the outcome for several other CARDIA manuscripts and Budoff et al. recently showed that a CAC score of 1–10 had a threefold higher risk for incident CHD events compared with CAC = 0 [24–29]. Data for covariates were collected by the CARDIA study at the year 7 examination in 1992, which was the baseline for this study. Details of measurement of low-density lipoprotein cholesterol (LDL-c), systolic blood pressure (SBP), and C-reactive protein (CRP) level have been previously described [21]. Participants were classified as having diabetes mellitus (DM) if they had fasting serum glucose >125 mg/dL or if they self-reported diabetes medication use. Smoking was classified as never, prior or current.

The CARDIA study did not collect data on thromboembolism at the baseline for this ancillary study, the CARDIA year 7 examination. However, data for history of transient ischemic attack and stroke were collected by CARDIA at the year 10 examination and six participants in this study's data set self-reported transient ischemic attack or stroke. They were excluded in a repeat set of analyses in case they might have met Sapporo criteria for antiphospholipid syndrome at year 7.

Connective Tissue Diseases Screening Questionnaire

Participants completed a self-administered Connective Tissue Diseases Screening Questionnaire during the CARDIA year 20 examination. The Connective Tissue Diseases Screening Questionnaire utilizes an algorithm for identifying potential connective tissue diseases based on American College of Rheumatology Criteria and published clinical criteria [30].

Statistical methods

Descriptive characteristics were compared across four gender–race specific groups. χ^2 (for categorical variables) or ANOVA (for continuous variables) tests were used to assess the statistical significance of differences in selected characteristics as well as in the prevalence (%) of positive autoantibodies across gender–race groups. Logistic regression was used to estimate odds ratios (OR) and 95 % confidence intervals (CIs) of CAC positivity measured at years 15 and 20 separately for each autoantibody with adjustment for age, LDL-c, body mass index (BMI), SBP, DM, smoking status, and CRP collected at year 7. Analyses were performed in all participants, then stratified by gender followed by race due to known differences in CAC and autoantibody positivity by gender and race. Separate analyses performed in the four race–gender groups (black women, black men, white women, white

men) demonstrated no effect modification, so the data presented are stratified by race or gender only.

The analyses were also repeated after excluding participants with a Connective Tissue Diseases Screening Questionnaire score indicating the probable presence of systemic lupus erythematosus, rheumatoid arthritis, Sjögren's syndrome, systemic sclerosis or mixed connective tissue disease. For the association of CAC with the number of anti- β 2-GPI isotypes, two dummy variables were employed, one positive isotype and two or three positive isotypes, with zero isotypes as the referent. Analyses were conducted using SAS version 9.2 (SAS Institute Inc, Cary, NC, USA).

Results

The demographic characteristics and baseline cardiovascular risk factors were compared across race and gender groups (Table 1). Black participants had a higher BMI and were more likely to be current smokers. Men had a higher serum LDL-c and SBP compared with women. CRP was higher in blacks than in whites and higher in women. There was a very low prevalence of DM given the young age of the study population. In order to contrast this study's participants with the baseline CARDIA study population, the demographic characteristics and baseline cardiovascular risk factors were compared with those of CARDIA participants who were not included in the current study due to unavailability of serum or unavailability of CAC. The CARDIA participants who were not included were more likely to be black and were more likely to be current smokers compared with the included participants (data not shown).

Prevalence of APA and coronary calcium

The prevalence of positive aCL and anti- β 2-GPI isotypes across race and gender groups is shown in Table 2. In general, women had a higher prevalence of a positive aCL and anti- β 2-GPI IgM than men. As has been previously shown by the CARDIA study [26], men had a higher prevalence of CAC >0 than women and white men had the highest prevalence (Fig. 2). The prevalence of CAC >0 was higher at year 20 than at year 15. This is expected given the higher prevalence of traditional cardiovascular risk factors as the CARDIA participants aged.

Associations between APA and coronary calcium

APA were clearly associated with sub-clinical atherosclerosis measured as CAC >0 at the CARDIA year 15 examination. IgG and IgA anti- β 2-GPI antibodies were associated with CAC >0 at year 15 in all participants after adjustment for traditional cardiovascular risk factors [IgG: OR 6.4 (95 % CI 2.4, 16.8); IgA: OR 5.6 (95 % CI 2.3, 13.2)] (Table 3). These associations also remained in analyses stratified by gender or race. Anti- β 2-GPI IgM was marginally associated with CAC >0 at year 15 [IgM: OR 1.7 (95 % CI 1.0, 3.1)] (Table 3), but the relationship was not present in men when the analyses were stratified by gender. aCL IgG was also associated with CAC >0 in all participants [OR 5.1 (95 % CI 1.4, 18.6)] and the associations were significant in men and black participants (Table 3). There was no

association between aCL IgM and CAC >0. (Due to a prevalence of zero for aCL IgA, its association with CAC was not assessed.)

Anti- β 2-GPI antibodies were associated with CAC >0 at year 20, but the relationship was not as strong as that for CAC at year 15. Anti- β 2-GPI IgG antibodies were associated with CAC >0 at year 20 in all participants [IgG: OR 3.1 (95 % CI 1.2, 7.7)]. Anti- β 2-GPI IgA and aCL IgG were marginally associated with CAC >0 at year 20 [anti- β 2-GPI IgA: OR 1.7 (95 % CI 0.7, 4.2), aCL IgG: OR 2.2 (95 % CI 0.7, 7.1)], but in the stratified analyses the relationship was not present in every group. There was no clear association between anti- β 2-GPI IgM or aCL IgM and CAC >0 at year 20 (Table 3).

We also assessed the association between APA and progression of CAC from year 15 to year 20. Progression was defined as either incident CAC at year 20 or an increase in CAC score of ≥ 20 between the year 15 and year 20 examinations. Anti- β 2-GPI IgG was associated with progression of CAC in all participants [OR 3.1 (95 % CI 1.1, 9.1)]. The association between anti- β 2-GPI IgG and progression of CAC was also significant in women [OR 4.2 (95 % CI 1.0, 17.0)] and in black participants [OR 5.8 (95 % CI 1.1, 29.0)] after stratification by gender and race. The trend for an association in men and white participants did not reach statistical significance. There was no association between anti- β 2-GPI IgM, anti- β 2-GPI IgA or any of the aCL isotypes and CAC progression.

To assess for a graded association between the number of anti- β 2-GPI isotypes and CAC >0, we measured the association between the presence of one isotype and then the presence of two or three isotypes and CAC >0 at year 15 and year 20. Participants with one positive anti- β 2-GPI isotype had modestly increased odds of CAC >0 at year 15 compared with participants without any anti- β 2-GPI isotypes [OR 1.4 (95 % CI 0.8, 2.6)] (Table 4). Participants with two or three positive anti- β 2-GPI isotypes had eightfold-increased odds of CAC >0 at year 15 compared with participants without any anti- β 2-GPI isotypes [OR 8.6 (95 % CI 3.3, 22.9)]. Table 4 demonstrates the graded association across groups stratified by gender and race, but the confidence intervals are wide due to the small numbers of participants with both CAC and two or three positive isotypes in the stratified analyses. The point estimates for the associations between number of isotypes and CAC >0 at year 20 were stronger for participants with two or three isotypes, but the findings did not reach statistical significance (Table 4). This analysis was not performed for aCL because no participants had two or three aCL isotypes.

We also measured the association between anti- β 2-GPI or aCL antibodies and CAC ≥ 20 as the outcome because this threshold is more specific for the presence of atherosclerosis, and our findings were similar in the repeat set of analyses. Given its high negative predictive value for the presence of prevalent connective tissue diseases, we used the results from the Connective Tissue Diseases Screening Questionnaire to identify participants who might have an active connective tissue disease [30]. There were 159 participants who had a Connective Tissue Diseases Screening Questionnaire score consistent with the probable presence of lupus, rheumatoid arthritis, Sjögren's syndrome, systemic sclerosis or mixed connective tissue disease, and our results were unchanged in sensitivity analyses conducted after excluding those participants. In addition, our results were unchanged in sensitivity

analyses conducted after excluding six participants who self-reported transient ischemic attack or stroke at the year 10 examination.

Discussion

This study found that anti- β 2-GPI IgG, anti- β 2-GPI IgA, and aCL IgG were associated with CAC at year 15 in all participants and in analyses stratified by gender or race. Anti- β 2-GPI IgM antibodies were marginally associated with CAC at year 15. The associations between anti- β 2-GPI or aCL antibodies and CAC at year 20 were not as strong. However, there was an association between anti- β 2-GPI IgG and new incidence or progression of CAC from year 15 to year 20. There also appeared to be a graded association with higher point estimates in those participants with two or more anti- β 2-GPI isotypes. There did not appear to be effect modification for the associations from gender or race.

The findings in this study are in alignment with previous studies identifying associations between antiphospholipid syndrome or APA and cardiovascular outcomes including coronary heart disease and intima media thickness. For instance, in the Hopkins Lupus Cohort, the presence of lupus anticoagulant was associated with myocardial infarction [18]. In a rheumatoid arthritis study, patients with elevated aCL were found to have higher carotid intima media thickness than patients without aCL [31]. In patients with primary antiphospholipid syndrome, participants over the age of 40 were found to have higher intima media thickness levels compared with controls [17], and aCL IgG correlated with intima media thickness in individuals with primary antiphospholipid syndrome or known positive APA [15]. In a non-rheumatic disease population, patients with myocardial infarction or angina had higher levels of aCL and anti- β 2-GPI antibodies compared with controls [5]. Similarly, in a case-control study, patients with myocardial infarction had higher APA prevalence and level, and APA level correlated with intima media thickness [16].

The finding of an association between APA and a subclinical measure of atherosclerosis in a young adult population suggests that the role of APA in the pathogenesis of atherosclerosis is not merely prothrombotic. There is evidence supporting the hypothesis that APA promote other autoimmune, non-thrombotic pathways in early atherosclerosis. For example, it was demonstrated that both monoclonal aCL bound to β 2-GPI and anti- β 2-GPI IgG derived from an antiphospholipid syndrome mouse model enhanced oxidized LDL/ β 2-GPI complex binding to macrophages [10, 11]. This demonstrates the pro-atherogenic property of APA, given that oxidized LDL uptake by macrophages promotes foam cell formation. Moreover, APA increased atherosclerotic lesions in a mouse model of atherosclerosis, the LDL-receptor knockout mouse (LDL-RKO): mouse aCL, induced by immunization of LDL-RKO mice with human aCL, led to increased fatty streak formation [12], and immunization of LDL-RKO mice with β 2-GPI also led to accelerated atherosclerotic lesions [13]. Subsequent studies demonstrated accelerated atherosclerosis after the adoptive transfer of β 2-GPI reactive lymphocytes [12]. Therefore, it is hypothesized that oxidized LDL/ β 2-GPI complexes are internalized by macrophages by anti- β 2-GPI-antibody-mediated phagocytosis [14].

Other investigators did not find such an association between APA and atherosclerosis: several studies failed to identify a relationship between aCL antibodies and intima media thickness [18, 32, 33] or CAC [5, 18, 19]. Moreover, participants with anti- β 2-GPI were not found to have higher intima media thickness or CAC scores [5, 32]. In contrast to the results reported herein, these studies did not find an association between APA and CAC. The difference between the studies may be because they had fewer participants, and, in most cases, the participants had active connective tissue diseases [18, 19, 32]. In addition, in some studies, the participants already had cardiac events and so, compared with CARDIA participants, they likely had a higher prevalence of traditional cardiovascular risk factors which may have accounted for the majority of their risk, thereby attenuating the relationships between APA and CAC. It is also possible that we found associations because our autoantibody measurement methods took steps to exclude nonspecific binding as a contributing factor.

One limitation of this study is that we were only able to measure APA at one time point due to limitations in the availability of CARDIA's serial serum samples. Given that APA vary over time, it would have been valuable to have multiple measures to assess autoantibody seroconversion at differing time points and to assess cross-sectional analyses with CAC at years 15 and 20. While we excluded participants with possible connective tissue diseases in sensitivity analyses and we did not find differences in the associations that we identified between APA and CAC, we were unable to adjust our analyses for immunosuppressive medication use, such as corticosteroids, which might have affected our results. Moreover, we were unable to assess the effect of time-varying traditional cardiovascular risk factors since autoantibodies were only measured at one time point. Therefore, future studies are needed to measure autoantibodies in stored serum from CARDIA years 15 and 20 in order to address these limitations.

The association between APA and CAC at the CARDIA year 20 examination was not as strong as the association measured 5 years earlier at the year 15 examination. This is not unexpected, given the higher prevalence of traditional cardiovascular risk factors which might play a more prevalent role in the pathogenesis of atherosclerosis as the CARDIA population ages. For example, participants had a higher prevalence of DM and higher CRP level and BMI at the year 20 examination than the year 15 examination (data not shown). It is also plausible that changes in autoimmune and inflammatory factors occurred during the long time frame between autoantibody testing at the year 7 examination and CAC measurement at year 20. For example, it is possible that factors leading to inflammation and autoimmunity, such as infectious or hormonal triggers, were present early on, accounting for the associations at year 15. By year 20, traditional risk factors were more prevalent and may have decreased the ability to identify the effects of the autoimmunity observed at year 15. Future studies are needed to measure autoantibodies at years 15 and 20 so that the cross-sectional associations between autoantibodies and CAC can be measured. In addition, the longitudinal association between autoantibodies at year 15 and CAC at year 20 could then be determined over a follow-up period similar to the longitudinal association between autoantibodies at year 7 and CAC at year 15. In order to confirm that the differences in findings between years 15 and 20 were not due to differences in the two data sets shown in Fig. 1, the associations between autoantibodies and CAC at years 15 and 20 were also

measured in the 1,515 participants with data for CAC at both CARDIA examinations, and the dilutional effect observed with year 20 CAC as the outcome was still present. Although autoimmunity measured at year 7 may have accounted for a smaller proportion of the atherosclerosis risk at year 20, the associations between anti- β 2-GPI IgG and progression of CAC from year 15 to year 20 indicate that autoimmunity still played a role in overall atherosclerosis at year 20.

This study is the first, to our knowledge, to report an association between APA and CAC in a cohort of community-based young adults. Further studies are needed for confirmation and to address the discrepancy between the findings for CAC measured early and later in life. Moreover, since this study demonstrated an association between APA measured in a low-risk young adult population and the subsequent development of CAC over 8 years, but less clearly over 13 years, one possible next step is to examine the association between autoantibodies measured at a closer time frame to the CARDIA year 20 examination. This would help to determine whether the differences found in the two data sets were related to the age of the study population and their prevalence of traditional cardiovascular risk factors, or to changes in autoimmune factors over time. Then, if these findings are confirmed in subsequent studies, basic science studies will be needed to further evaluate the role of APA in the pathogenesis of atherosclerosis.

Most importantly, if further studies also demonstrate a relationship between APA in young adults and the subsequent development of atherosclerosis later in life, then screening measures to identify young adults with positive APA may be warranted. APA, as well as other rheumatic disease-related autoantibodies, have been identified in the serum before the onset of clinical thrombosis or rheumatic disease [34–37]. In individuals found to have APA, measures to prevent their thrombotic effects are often used in clinical practice [35, 36]. Moreover, studies are in progress to characterize autoimmune rheumatic disease risk in individuals with pre-clinical autoantibodies so that primary prevention strategies might be implemented to prevent clinical autoimmune sequelae [37]. It is plausible that young adults with APA might be at risk for coronary heart disease, and might benefit from prevention strategies designed to attenuate autoimmune processes. Additionally, young adults found to have APA might benefit from screening for traditional cardiovascular disease risk factors so that they might be targeted for risk factor modification.

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Fig. 1.

Selection of study participants. 2,203 participants were included. 1,838 were analyzed in the data set with year 15 CAC as the outcome and 1,880 were analyzed in the data set with year 20 CAC as the outcome. 1,515 participants were analyzed in both data sets because they had data for CAC at both years 15 and 20

Fig. 2.

Percent of participants with CAC > 0 in race and gender groups by examination year

Table 1

Descriptive characteristics of the study samples selected from the CARDIA Study

Year 7 (1992) characteristics ^a	Black women	Black men	White women	White men	P value [*] , 2-sided
Participants with data for CAC year 15 and autoantibodies year 7					
No.	489	331	495	523	
Age (years)	31.7	31.8	32.7	32.6	<0.001
Smoking status (%)					<0.001
Non-smoking	58.9	58.6	57.8	62.7	
Ex-smokers	12.9	9.7	25.1	16.8	
Current smokers	28.2	31.7	17.2	20.5	
BMI (kg/m ²)	28.7	27.1	24.8	26.0	<0.001
LDL-c (mg/dl)	105.3	113.3	102.7	116.3	<0.001
SBP (mm Hg)	108.7	113.9	101.3	110.2	<0.001
CRP (mg/L)	4.1	2.3	2.5	1.6	<0.001
Diabetes mellitus (%)	1.6	1.8	0.2	1.0	0.083
Participants with data for CAC year 20 and autoantibodies year 7					
No.	524	303	535	518	
Age (years)	31.5	31.9	32.8	32.5	<0.001
Smoking status (%)					<0.001
Non-smoking	60.3	58.8	56.8	60.6	
Ex-smokers	11.5	9.6	26.0	17.2	
Current smokers	28.2	31.7	17.2	22.2	
BMI (kg/m ²)	28.5	26.9	24.6	26.0	<0.001
LDL-c (mg/dl)	104.8	112.4	102.1	115.7	<0.001
SBP (mm Hg)	108.2	113.2	101.5	110.3	<0.001
CRP (mg/L)	4.0	2.4	2.4	1.7	<0.001
Diabetes mellitus (%)	1.3	2.0	0.4	1.0	0.154

BMI body mass index, *CAC* coronary artery calcification, *CARDIA* Coronary Artery Risk Development in Young Adults, *CRP* C-reactive protein, *LDL-c* low density lipoprotein, *SBP* systolic blood pressure

* *P* value for overall group comparison based on χ^2 or *F* test

^aData are means, unless otherwise indicated

Table 2

Percent positive CTD-related autoantibodies in study samples selected from the CARDIA Study by race and gender

CTD-related autoantibodies	Black women	Black men	White women	White men	<i>P</i> value*, 2-sided
Participants with data for CAC year 15 and autoantibodies year 7					
No.	489	331	495	523	
aCL IgM	1.8	0.9	2.2	1.2	0.373
aCL IgG	1.4	1.8	0.8	0.2	0.075
aCL IgA	0	0	0	0	NA
Anti-β2-GPI IgM	6.8	4.5	9.9	6.5	0.023
Anti-β2-GPI IgG	1.4	1.2	1.2	1.3	0.989
Anti-β2-GPI IgA	1.6	2.4	1.4	1.9	0.743
Participants with data for CAC year 20 and autoantibodies year 7					
No.	524	303	535	518	
aCL IgM	1.9	1.0	1.5	1.4	0.751
aCL IgG	1.7	1.7	0.6	0.4	0.073
aCL IgA	0	0	0	0	NA
Anti-β2-GPI IgM	6.7	5.0	8.6	7.0	0.248
Anti-β2-GPI IgG	1.5	1.0	1.1	1.4	0.900
Anti-β2-GPI IgA	2.1	2.3	1.3	1.5	0.647

Anti-β2-GPI anti-β2 glycoprotein I, *aCL* anti-cardiolipin, *CAC* coronary artery calcification, *CARDIA* Coronary Artery Risk Development in Young Adults, *CTD* connective tissue disease, *IgA* immunoglobulin A, *IgG* immunoglobulin G, *IgM* immunoglobulin M, *NA* not applicable

* *P* value for overall group comparison based on χ^2 test

Table 3

Adjusted odds ratios (95 % confidence interval) for CAC >0 by autoantibody positivity in study samples from the CARDIA Study

CTD-related autoantibodies	All participants			Women			Men			Black			White		
	OR	95 % CI	OR	95 % CI	OR	95 % CI	OR	95 % CI	OR	95 % CI	OR	95 % CI	OR	95 % CI	
Odds ratios (95 % CI) for year 15 CAC ≥0 by autoantibody positivity															
No.	1838				984				854				820		1018
aCL IgM	0.8	0.2, 3.6	1.3	0.2, 10.6	0.5	0.1, 4.2	NA						1.0	0.2, 5.1	
aCL IgG	5.1	1.4, 18.6	1.8	0.2, 15.6	10.4	1.6, 68.0	5.0	1.1, 23.4	3.9	0.3, 47.3					
Anti-β2-GPI IgM	1.7	1.0, 3.1	3.3	1.4, 7.5	1.0	0.4, 2.3	1.8	0.7, 5.2	1.7	0.8, 3.4					
Anti-β2-GPI IgG	6.4	2.4, 16.8	7.4	1.8, 30.6	5.5	1.4, 22.3	5.0	1.1, 23.8	7.2	2.0, 25.5					
Anti-β2-GPI IgA	5.6	2.3, 13.2	10.3	2.8, 37.2	4.1	1.2, 13.7	7.4	2.0, 27.1	4.2	1.3, 13.9					
Odds ratios (95 % CI) for year 20 CAC ≥0 by autoantibody positivity															
No.	1,880				1,059				821				827		1053
aCL IgM	0.8	0.3, 2.7	2.6	0.7, 9.6	0.2	0.02, 1.8	1.9	0.4, 9.9	0.5	0.1, 2.4					
aCL IgG	2.2	0.7, 7.1	0.8	0.1, 6.7	3.7	0.7, 20.4	2.3	0.6, 8.8	1.5	0.1, 16.4					
Anti-β2-GPI IgM	0.9	0.5, 1.5	1.3	0.6, 2.7	0.7	0.4, 1.5	1.1	0.5, 2.6	0.8	0.4, 1.5					
Anti-β2-GPI IgG	3.1	1.2, 7.7	5.7	1.8, 18.2	1.6	0.4, 5.8	4.2	1.1, 16.5	2.4	0.7, 8.3					
Anti-β2-GPI IgA	1.7	0.7, 4.2	2.3	0.6, 8.3	1.4	0.4, 4.9	2.6	0.8, 8.5	0.8	0.2, 3.7					

Adjusted for year 7 age, race (except for race-specific groups), gender (except for gender-specific groups), LDL-c, BMI, SBP, smoking status, DM, and CRP
 Bold values indicate the statistical significance

Anti-β2-GPI anti-β2 glycoprotein I, *aCL* anti-cardiolipin, *CAC* coronary artery calcification, *CARDIA* Coronary Artery Risk Development in Young Adults, *CI* confidence interval, *CTD* connective tissue disease, *IgA* immunoglobulin A, *IgG* immunoglobulin G, *IgM* immunoglobulin M, *NA* not applicable, *OR* odds ratio

Table 4

Adjusted odds ratios (95 % CI) for CAC >0 by number of anti-β2-GPI isotypes in study samples from the CARDIA Study

Number of anti-β2-GPI isotypes	All participants			Men			Black			White		
	OR	95 % CI	OR	95 % CI	OR	95 % CI	OR	95 % CI	OR	95 % CI	OR	95 % CI
CAC >0 year 15 as outcome												
0 isotypes	1	Reference	1	Reference	1	Reference	1	Reference	1	Reference	1	Reference
1 positive isotype	1.4	0.8, 2.6	2.6	1.1, 6.4	1.0	0.5, 2.3	1.0	0.3, 3.1	1.6	0.8, 3.3		
2 positive isotypes	8.6	3.3, 22.9	12.6	3.1, 50.7	7.3	1.7, 31.8	17.7	3.4, 92.4	6.4	1.9, 21.9		
CAC >0 year 20 as outcome												
0 isotypes	1	Reference	1	Reference	1	Reference	1	Reference	1	Reference		
1 positive isotype	1.0	0.6, 1.7	1.6	0.8, 3.3	0.8	0.4, 1.5	1.3	0.6, 2.8	0.8	0.4, 1.6		
2 positive isotypes	2.0	0.7, 5.4	2.5	0.6, 9.6	1.5	0.3, 6.6	3.2	0.7, 14.6	1.3	0.3, 5.2		

Adjusted for year 7 age, race (except for race-specific groups), gender (except for gender-specific groups), LDL-c, BMI, SBP, smoking status, DM, and CRP
Bold values indicate the statistical significance
Anti-β2-GPI anti-β2 glycoprotein I, CAC coronary artery calcification, CARDIA Coronary Artery Risk Development in Young Adults, CI confidence interval, OR odds ratio