Lipoprotein(a) Complexes with Beta2-Glycoprotein I in Patients with Coronary Artery Disease

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Aim: To investigate the possible mechanisms and association of increased complexes of β2-glycoprotein I with lipoprotein(a) [β2-GPI-Lp(a)] levels with the presence and extent of coronary artery disease (CAD).

Methods: β2-GPI-Lp(a) levels were measured in 116 patients with acute coronary syndromes (ACS), 72 patients with stable CAD and 100 control subjects.

Results: Compared to the control, β2-GPI-Lp(a) levels (expressed after logarithmically transformation: ACS, 0.22±0.45 U/mL; stable CAD, 0.05±0.55 U/mL; control, −0.31±0.61 U/mL) significantly increased in both patients with ACS (p<0.001) and stable CAD (p<0.001). Univariate logistic regression analysis of risk factors revealed that the presence of β2-GPI-Lp(a), ox-Lp(a) or Lp(a) was a strong risk factor for stable CAD [β2-GPI-Lp(a), OR 3.17, 95% CI 1.65, 6.07; ox-Lp(a), OR 2.54, 95% CI 1.33, 4.85; Lp(a), OR 2.50, 95% CI 0.95, 6.16; Lp(a), OR 3.00, 95% CI 1.56, 5.75; respectively], and especially for ACS [β2-GPI-Lp(a), OR 5.38, 95% CI 2.57, 7.49; ox-Lp(a), OR 7.55, 95% CI 4.12, 13.84; Lp(a), OR 4.33, 95% CI 2.56, 7.80; respectively]. In multivariate analysis, adjusting for age, sex and plasma lipid levels, the presence of β2-GPI-Lp(a) or Lp(a) was a risk factor for both stable CAD and ACS. Ox-Lp(a) was a risk factor only for ACS, while not for stable CAD. β2-GPI-Lp(a) levels were found to be positively associated with Lp(a), ox-Lp(a), maximal stenosis and a number of vessel diseases in patients with ACS or stable CAD, respectively. Multiple linear regression analysis found that ox-Lp(a) and maximal stenosis accounted for 46.2% of the variation in β2-GPI-Lp(a) levels.

Conclusions: Elevated levels of β2-GPI-Lp(a) are associated with the presence and severity of CAD, and may be a strong risk factor for atherosclerosis.


Key words; Atherosclerosis, Coronary artery disease, Lipoproteins, Oxidation, β2-glycoprotein I

Introduction

Lipoprotein(a) [Lp(a)] is an atherogenic particle that structurally resembles a low density lipoprotein (LDL) particle but contains a molecule of apolipoprotein(a) [apo(a)] attached to apoB-100 by a disulfide bond11. Elevated plasma concentrations of Lp(a) have been shown to be one of the independent risk factors for atherosclerosis2-4. Oxidized Lp(a) [ox-Lp(a)] has been reported to play a greater role than native Lp(a) in atherosclerosis. Ox-Lp(a) is a ligand for scavenger receptors5, 6 and might reasonably be expected to contribute to foam cell formation. Ox-Lp(a) may also induce adhesion molecular expression on monocytes, promoting their recruitment and adhesion to the endothelium7, and influence the responsiveness of platelets to various agonists8. Modified forms of Lp(a), some resembling ox-Lp(a), have been identified in human atheromatous lesions9. In addition, in vitro oxidative modification increases the
inhibitory effect of Lp(a) on plasminogen binding to cell surfaces, which could attenuate fibrinolytic activity by reducing plasminogen activation. Ox-Lp(a) levels have been reported to be present in newborns and children, and increased in rheumatoid arthritis patients with excessive cardiovascular events. Furthermore, elevated ox-Lp(a) levels have been found to be associated with the presence of stable coronary artery disease (CAD) and acute coronary syndromes (ACS), and with the severity of ACS.

In vitro studies have demonstrated that beta-2-glycoprotein I (β2-GPI) bound Lp(a) with high affinity, suggesting that β2-GPI might bind Lp(a) to form complexes of β2-GPI with Lp(a) [β2-GPI-Lp(a)] in vivo. Our recent study found for the first time that β2-GPI-Lp(a) existed in the circulation. Significantly increased β2-GPI-Lp(a) concentrations were also found in systemic lupus erythematosus (SLE) patients with excessive cardiovascular events and might act as an additional predictor of premature atherosclerosis.

It has also been reported that β2-GPI levels increase on isolated Lp(a) from CAD patients; therefore, we hypothesized that β2-GPI-Lp(a) may be associated with the presence and severity of CAD, and be a strong risk factor for atherosclerosis. Hence we designed this study to evaluate the clinical value of β2-GPI-Lp(a) levels in stable and unstable coronary syndromes.

Methods

Subjects

The present study included 116 patients with ACS, 72 patients with stable CAD, and 100 control subjects. The control subjects were randomly selected from 500 healthy subjects who had visited Jinling Hospital for routine health examination from January to February 2010, and were found to be normal on physical and electrocardiography and laboratory tests, and without diseases such as hyperlipemia, hypertension, diabetes mellitus, or any clinical evident sign of atherosclerosis. All the subjects were living in Nanjing, China and belonged to the Han population, the major ethnic group in China.

The CAD patients in this study were consecutively selected from patients admitted to the Department of Cardiology of Jinling Hospital between January and December 2010, who were undergoing clinically indicated coronary angiography. Angiograms of all the CAD patients showed at least 50% stenosis of 1, 2, or 3 coronary arteries. One hundred and sixteen patients with ACS included acute myocardial infarction patients and unstable angina with Braunwald classification II or III. Seventy-two patients with angiographically documented CAD and no cardiac events/procedures for more than 1 year were considered to have stable CAD. The exclusion criteria of the CAD patients included prior coronary revascularization and the presence of renal disease. In patients with ACS, blood samples were taken on admission. Blood samples were collected at least 12 h after fasting from control subjects and patients with stable CAD. The blood sample was collected into a tube containing EDTA (1 mg/mL) and plasma was separated immediately and stored at −70°C until analysis. All laboratory assays were conducted within 1 year of blood sampling. This study protocol was approved by the Ethics Committee of Jinling Hospital, and all the subjects provided written informed consent.

Angiographic Analysis

Catheterization was performed by either the Sones or the Judkins technique. Multiple views including angulated views were obtained, and the angiograms were evaluated. Maximal stenosis in each coronary artery segment was assessed by a cardiologist, who was unaware of the risk factors, with the use of handheld calipers or by visual analysis according to the segmental classification system of the Coronary Artery Surgery Study. The extent of angiographically documented CAD was quantified in the left anterior descending coronary artery, the left circumflex artery, or the right coronary artery as follows: normal coronary arteries (smooth, with either no stenosis or stenosis of <10% of the luminal diameter), mild disease (stenosis of 10% to 50% of the luminal diameter in ≥1 coronary artery), or 1, 2, or 3-vessel disease, defined as stenosis of more than 50% of the luminal diameter.

Assays

β2-GPI-Lp(a) was measured by a sandwich enzyme-linked immunosorbent assay (ELISA), using anti-human β2-GPI antibody as the capture antibody and detection with polyclonal antibody against apo(a) enzyme conjugate as previously described. In brief, β2-GPI-Lp(a) was precipitated with polyethylene glycol (PEG). Five hundred microliters of plasma was first incubated with MgCl₂ (final concentration 10 μmol/L) at 37°C for 2 h, and then PEG-6000 (Sigma) was added to isolate β2-GPI-Lp(a) from the endogenous free form of β2-GPI and Lp(a) to eliminate its possible influence on the assay. The samples were incubated overnight at 4°C and then centrifuged at 10,000 rpm for 20 min. The precipitates were resuspended in 500 μL washing solution containing 0.5% gelatin and 0.05% Tween-20 in 0.01 mol/L PBS.
pooled fresh-frozen plasma sample (mixed plasma from 50 healthy subjects) was used as the reference serum of \( \beta_2 \)-GPI-Lp(a). \( \beta_2 \)-GPI-Lp(a) was also precipitated every time as above, and the value of \( \beta_2 \)-GPI-Lp(a) was expressed as 1 relative absorbance unit (U/mL).

Ox-Lp(a) was measured by sandwich ELISA, using a polyclonal antibody against oxidized LDL (ox-LDL) as the capture antibody and detection with monoclonal anti-apo(a) enzyme conjugate as previously described. Antibodies to ox-LDL were obtained by immunization of New Zealand white female rabbits with ox-LDL. The resulting rabbit antiserum was first fractionated by affinity chromatography with immobilized protein G, and the IgG fractions were then absorbed in a column of immobilized native LDL. Washout from the column of immobilized native LDL, containing antibodies to ox-LDL and irrelevant IgG, was used to capture ox-LDL, which had no reactivity with native LDL. A pooled fresh-frozen plasma sample (mixed plasma from 50 healthy subjects) was used as the reference plasma of ox-Lp(a). The value of ox-Lp(a) was determined by ELISA repeatedly, based on the concentration of copper ion oxidized Lp(a) as the standard.

Lp(a) concentration was also detected by sandwich ELISA as previously described. Using a monoclonal anti-apo(a) as the capture antibody, bound Lp(a) particles were detected with polyclonal anti-apo(a) enzyme conjugates. Polyclonal sheep anti-apo(a) was developed in the laboratory. Antibodies against LDL, plasminogen and other apolipoproteins in Lp(a) antisera were removed by absorbing on an affinity chromatographic column of Sepharose 4B (Pharmacia) coupled with plasminogen, and pooled sera obtained from Lp(a)-negative subjects. Reference serum of Lp(a) was from Immuno AG Vienna. Total cholesterol, triglyceride, high density lipoprotein (HDL) cholesterol (Daiichi Pure Chemicals, Japan) were measured on a Hitachi 7600 analyzer. LDL cholesterol was estimated using the Friedewald formula.

Statistical Analysis

Statistical analyses were performed with SPSS 16.0 and the values are expressed as the mean ± standard deviation. Lp(a), ox-Lp(a), \( \beta_2 \)-GPI-Lp(a) and triglyceride concentrations of non-normal distribution were logarithmically transformed. The differences of variants among groups were analyzed by one-way analysis of variance (ANOVA), and the differences between groups were subsequently determined by Fisher’s LSD test when appropriate. The difference in the extent of angiographically documented disease between two groups was analyzed by Student’s \( t \)-test.

The differences in sex among groups were analyzed by the Chi-square test. As an approximation of the relative risk, the odds ratio (OR) and 95% confidence interval (CI) were calculated for several putative risk factors with univariate and multivariate logistic regression analysis, in which the values of the 75th percentile of each risk factor in the controls were chosen as the cut-off point. All study subjects were categorized into the stable CAD, ACS or control group, which was treated as a dependent three-category variable. In univariate analysis, each model included only one variable, \( \beta_2 \)-GPI-Lp(a), ox-Lp(a) or Lp(a), respectively. In multivariate analysis, a model included all three variables; age, sex and plasma lipid levels were controlled. Correlations between variables were calculated by the non-parametric Spearman rank coefficient test. Multiple linear regression analysis were used to estimate the associations of \( \beta_2 \)-GPI-Lp(a) with Lp(a), ox-Lp(a), degree of CAD, age, sex and plasma lipid levels. \( p < 0.05 \) was considered significant.

Results

Baseline Clinical Characteristics and Lipid Levels in the Study Group

The baseline clinical characteristics of the patients and control subjects, indications for coronary angiography, and lipid measurements are showed in Table 1. The distributions of Lp(a), ox-Lp(a) and \( \beta_2 \)-GPI-Lp(a) levels in all the studied patients and subjects (\( n = 288 \)) were skewed toward lower values.

Association with Presence of ACS and Stable CAD

Compared to control subjects, \( \beta_2 \)-GPI-Lp(a) and ox-Lp(a) levels were found to be significantly increased in both patients with ACS and stable CAD, and Lp(a) levels were found to be increased only in ACS. Furthermore, ox-Lp(a) levels were significant higher in ACS than in stable CAD (Table 2).

The absolute values of Lp(a), ox-Lp(a) and \( \beta_2 \)-GPI-Lp(a) were not normally distributed. The values of the 75th percentile in the controls were chosen as the cut-off point for each trait. Cut-off values for Lp(a), ox-Lp(a) and \( \beta_2 \)-GPI-Lp(a) were 164.00 mg/L, 10.85 mg/L and 1.44 U/mL, respectively. \( \beta_2 \)-GPI-Lp(a), ox-Lp(a) and Lp(a) were significantly more present in stable CAD patients [\( \beta_2 \)-GPI-Lp(a), 50.0%, \( p = 0.001 \); ox-Lp(a), 45.8%, \( p = 0.004 \); Lp(a), 48.6%, \( p = 0.001 \); respectively] and ACS patients [\( \beta_2 \)-GPI-Lp(a), 62.9%, \( p = 0.000 \); ox-Lp(a), 71.6%, \( p = 0.000 \); Lp(a), 57.8%, \( p = 0.000 \); respectively] than in the control (all 25%). The results of univariate analysis of risk factors for stable CAD and ACS are shown in Table 3,
**Table 1.** Angiographic characteristics and lipid levels in the study groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Stable CAD (n=72)</th>
<th>ACS (n=116)</th>
<th>Control (n=100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>65.2 ± 9.8</td>
<td>62.7 ± 12.3</td>
<td>62.1 ± 9.2</td>
</tr>
<tr>
<td>Male/female</td>
<td>49/23</td>
<td>78/38</td>
<td>67/33</td>
</tr>
<tr>
<td>Angiographic analysis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximal stenosis (%)</td>
<td>0.79 ± 0.16</td>
<td>0.85 ± 0.20</td>
<td></td>
</tr>
<tr>
<td>Number of vessel diseases</td>
<td>1.85 ± 0.90</td>
<td>1.96 ± 0.91</td>
<td></td>
</tr>
<tr>
<td>Lipid levels</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.08 ± 1.09 *</td>
<td>5.31 ± 1.13 **</td>
<td>4.71 ± 0.67</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>3.02 ± 0.97 *</td>
<td>3.19 ± 1.05 **</td>
<td>2.42 ± 0.58</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.18 ± 0.23 **</td>
<td>1.19 ± 0.24 **</td>
<td>1.59 ± 0.22</td>
</tr>
<tr>
<td>log10 Triglycerides (mmol/L)</td>
<td>0.23 ± 0.23 *</td>
<td>0.24 ± 0.25 **</td>
<td>0.16 ± 0.17</td>
</tr>
</tbody>
</table>

Data are presented as the mean ± SD or number (% of subjects.
Compared with control: *p<0.05; **p<0.01.

**Table 2.** β₂-GPI-Lp(a), ox-Lp(a) and Lp(a) Levels in the Study Group

<table>
<thead>
<tr>
<th>Variable</th>
<th>Stable CAD (n=72)</th>
<th>ACS (n=116)</th>
<th>Control (n=100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>log10 Lp(a) (mg/L)</td>
<td>2.13 ± 0.35</td>
<td>2.23 ± 0.31 **</td>
<td>2.12 ± 0.22</td>
</tr>
<tr>
<td>log10 Ox-Lp(a) (mg/L)</td>
<td>0.93 ± 0.46 **</td>
<td>1.25 ± 0.39 ***</td>
<td>0.75 ± 0.39</td>
</tr>
<tr>
<td>log10 β₂-GPI-Lp(a) (U/mL)</td>
<td>0.05 ± 0.55 ***</td>
<td>0.22 ± 0.45 ***</td>
<td>−0.31 ± 0.61</td>
</tr>
</tbody>
</table>

Compared with control: *p<0.05; **p<0.01; ***p<0.001; compared with stable CAD: §p<0.001.

respectively. In these analyses, all values were analyzed as positive or negative irrespective of their concentrations. Logistic regression analysis of risk factors revealed that the presence of β₂-GPI-Lp(a), ox-Lp(a) or Lp(a) was a strong risk factor for stable CAD [β₂-GPI-Lp(a), OR 3.17, 95% CI 1.65, 6.07; ox-Lp(a), OR 2.54, 95% CI 1.33, 4.85; Lp(a), OR 3.00, 95% CI 1.56, 5.75; respectively], and especially for ACS [β₂-GPI-Lp(a), OR 5.38, 95% CI 2.97, 9.74; ox-Lp(a), OR 7.55, 95% CI 4.12, 13.84; Lp(a), OR 4.33, 95% CI 2.40, 7.80; respectively].

In multivariate analysis, adjusting for age, sex and plasma lipid levels, the presence of β₂-GPI-Lp(a) or Lp(a) was revealed to be a risk factor for both stable CAD [β₂-GPI-Lp(a), OR 9.13, 95% CI 2.24, 37.27; Lp(a), OR 7.61, 95% CI 2.11, 27.42; respectively] and ACS [β₂-GPI-Lp(a), OR 7.74, 95% CI 1.93, 31.13; Lp(a), OR 9.83, 95% CI 2.80, 34.53; respectively]. Ox-Lp(a) was found to be a risk factor only for ACS (OR 8.02, 95% CI 2.10, 30.63), while not for stable CAD (Table 4).

**Association with Lipid Parameters, Extent of Angiographically Documented Disease**

To study the relationship of β₂-GPI-Lp(a) with Lp(a), ox-Lp(a), lipid parameters, and the extent of CAD, simple linear correlation and multiple linear regression analysis were performed in patients with ACS and stable CAD, respectively. β₂-GPI-Lp(a) levels were found to be positively associated with Lp(a), ox-Lp(a), maximal stenosis and the number of vessel disease in both patients with ACS and stable CAD, respectively. β₂-GPI-Lp(a) levels were also found to be positively associated with LDL and HDL cholesterol in ACS. Similarly, Lp(a) was found positively associated with ox-Lp(a), β₂-GPI-Lp(a) in both stable CAD and ACS, and with LDL cholesterol and the number of vessel diseases only in ACS. Ox-Lp(a) was found to be positively associated with Lp(a), β₂-GPI-Lp(a) and the number of vessel diseases in both stable and ACS, respectively. The associations of ox-Lp(a) with triglyceride, HDL cholesterol and maximal stenosis were also noted in either stable or ACS (Table 5).

We next performed multiple linear regression analysis for β₂-GPI-Lp(a) versus each factor. A forward elimination procedure of stepwise analysis was used; the extent of CAD, Lp(a), ox-Lp(a), lipid parameters, age and sex were treated as independent variables, respectively. For similar associations of β₂-GPI-Lp(a) noted with lipid parameters and the extent of
CAD in stable CAD and ACS, we combined stable CAD and ACS for analysis (n = 182). Consequently, ox-Lp(a) and maximal stenosis were found to account for 46.2% of the variation in the concentration of \( \beta_2 \)-GPI-Lp(a) (Table 6).

### Discussion

This study shows for the first time that plasma \( \beta_2 \)-GPI-Lp(a) levels are associated with the presence and extent of angiographically documented CAD. Compared to control subjects, the plasma levels of \( \beta_2 \)-GPI-Lp(a) significantly increased in both patients with ACS and stable CAD. Associations among \( \beta_2 \)-GPI-Lp(a), ox-Lp(a) and degree of CAD were also found.

Lp(a) is considered to be an atherogenic lipoprotein; however, the mechanisms by which it contributes to atherosclerosis remain widely unknown. Recently, oxidized Lp(a) has been reported to have additional specific biological properties and to play a more potent role in atherosclerosis than native Lp(a)\(^{5-10}\). \( \beta_2 \)-GPI is characterized by its ability to bind lipoproteins, prothrombin, lecithin and other negatively charged materials\(^{23-26}\). In vitro studies have demonstrated that \( \beta_2 \)-GPI bound Lp(a) with high affinity\(^{16, 17}\), suggesting that \( \beta_2 \)-GPI might bind Lp(a) to form complexes in vivo. Our previous study found that \( \beta_2 \)-GPI-Lp(a) indeed existed in serum samples, and that \( \beta_2 \)-GPI-Lp(a) as well as ox-Lp(a) concentrations were significantly elevated in SLE patients\(^{18}\). SLE is associated with premature atherosclerosis, and the increased susceptibility of plasma lipids and lipoproteins to oxidation could at least in part explain the pathogenesis\(^{27, 28}\).

It has also been reported that \( \beta_2 \)-GPI levels increase on isolated Lp(a) from CAD patients\(^{19}\). We hypothesize that \( \beta_2 \)-GPI-Lp(a), just like ox-Lp(a), might be a key risk factor for atherosclerosis. Thus, it is noteworthy to investigate the role of \( \beta_2 \)-GPI-Lp(a) in the pathogenesis of atherosclerosis. In the present study, \( \beta_2 \)-GPI-Lp(a) levels were found to be increased in both patients with ACS and stable CAD.
more, univariate logistic regression analysis of risk factors revealed that the presence of $\beta_2$-GPI-Lp(a), ox-Lp(a) or Lp(a) was a strong risk factor for stable CAD, and especially for ACS. In multivariate analysis, adjusting for age, sex and plasma lipid levels, the presence of $\beta_2$-GPI-Lp(a) or Lp(a) was still found to be a risk factor for both stable CAD and ACS. Ox-Lp(a) was a risk factor for ACS, while not for stable CAD. The clinical values of ox-Lp(a) and Lp(a) are similar to those we previously reported\(^{13}\). These results imply that $\beta_2$-GPI-Lp(a) complexes might act as an additional predictor of atherosclerosis.

A series of studies have demonstrated convincingly that a key oxidized phospholipid (ox-PL) is preferentially associated with Lp(a)\(^{29}\) and correlates with both the presence and extent of angiographically documented CAD\(^{30,31}\), and their concentrations increase after ACS\(^{32}\) and immediately after percutaneous coronary intervention (PCI)\(^{33}\). An important role in the accumulation of ox-PL on Lp(a) may be also played by $\beta_2$-GPI, which binds to the kringle IV domain of apo(a)\(^{16}\), as well as to anionic phospholipids and ox-PL\(^{34}\). Importantly, high $\beta_2$-GPI levels were also found on the Lp(a) of CAD patients, whereas removal of apo(a) from the Lp(a) particles of these patients led to a reduction of $\beta_2$-GPI levels and an increase in lipoprotein-associated phospholipase A\(_2\) (Lp-PLA\(_2\)) catalytic efficiency. Substrates for Lp-PLA\(_2\) contain oxidatively fragmented residues at the sn-2 position (ox-PL). Thus, higher amounts of $\beta_2$-GPI on the Lp(a) of CAD patients could contribute to the seques-

Table 5. Correlations coefficient between Lp(a), ox-Lp(a), $\beta_2$-GPI-Lp(a), the extent of CAD, and lipid parameters in stable CAD and ACS

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total Cholesterol</th>
<th>Triglyceride</th>
<th>HDL Cholesterol</th>
<th>LDL Cholesterol</th>
<th>Lp(a)</th>
<th>Ox-Lp(a)</th>
<th>Lp(a)-$\beta_2$-GPI</th>
<th>Maximal stenosis</th>
<th>Number of vessel diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stable CAD</strong></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Lp(a)</td>
<td>0.141</td>
<td>-0.161</td>
<td>0.157</td>
<td>0.206</td>
<td>0.522</td>
<td>0.403</td>
<td>0.119</td>
<td>0.179</td>
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<tr>
<td></td>
<td>$p=0.236$</td>
<td>$p=0.177$</td>
<td>$p=0.187$</td>
<td>$p=0.082$</td>
<td>$p=0.000$</td>
<td>$p=0.000$</td>
<td>$p=0.319$</td>
<td>$p=0.132$</td>
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<tr>
<td>Ox-Lp(a)</td>
<td>0.119</td>
<td>-0.254</td>
<td>0.188</td>
<td>0.205</td>
<td>0.522</td>
<td>0.643</td>
<td>0.179</td>
<td>0.270</td>
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<tr>
<td></td>
<td>$p=0.320$</td>
<td>$p=0.031$</td>
<td>$p=0.115$</td>
<td>$p=0.084$</td>
<td>$p=0.000$</td>
<td>$p=0.000$</td>
<td>$p=0.133$</td>
<td>$p=0.022$</td>
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<tr>
<td>$\beta_2$-GPI-Lp(a)</td>
<td>0.058</td>
<td>-0.211</td>
<td>0.190</td>
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<td>0.403</td>
<td>0.643</td>
<td>0.263</td>
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<td></td>
<td>$p=0.628$</td>
<td>$p=0.075$</td>
<td>$p=0.111$</td>
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<td>$p=0.000$</td>
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<td>$p=0.026$</td>
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<tr>
<td>Lp(a)</td>
<td>0.214</td>
<td>-0.049</td>
<td>0.180</td>
<td>0.304</td>
<td>0.356</td>
<td>0.317</td>
<td>0.083</td>
<td>0.193</td>
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<tr>
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<td>$p=0.021$</td>
<td>$p=0.600$</td>
<td>$p=0.053$</td>
<td>$p=0.001$</td>
<td>$p=0.000$</td>
<td>$p=0.001$</td>
<td>$p=0.377$</td>
<td>$p=0.038$</td>
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<tr>
<td>Ox-Lp(a)</td>
<td>0.039</td>
<td>-0.120</td>
<td>0.233</td>
<td>0.105</td>
<td>0.356</td>
<td>0.694</td>
<td>0.223</td>
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<tr>
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<td>$p=0.680$</td>
<td>$p=0.199$</td>
<td>$p=0.012$</td>
<td>$p=0.261$</td>
<td>$p=0.000$</td>
<td>$p=0.000$</td>
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<td>$\beta_2$-GPI-Lp(a)</td>
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<td>0.193</td>
<td>0.317</td>
<td>0.694</td>
<td>0.335</td>
<td>0.199</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$p=0.121$</td>
<td>$p=0.599$</td>
<td>$p=0.005$</td>
<td>$p=0.038$</td>
<td>$p=0.001$</td>
<td>$p=0.000$</td>
<td>$p=0.000$</td>
<td>$p=0.032$</td>
<td></td>
</tr>
</tbody>
</table>

Table 6. Multiple linear regression models predicting $\beta_2$-GPI-Lp(a)

<table>
<thead>
<tr>
<th>Outcome variable/model</th>
<th>Beta</th>
<th>SE $\beta$</th>
<th>$p$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta_2$-GPI-Lp(a)</td>
<td>0.643</td>
<td>0.063</td>
<td>0.000</td>
<td>0.462</td>
</tr>
<tr>
<td>Ox-Lp(a)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximal stenosis</td>
<td>0.136</td>
<td>0.149</td>
<td>0.016</td>
<td>0.462</td>
</tr>
</tbody>
</table>

Multiple linear regression analysis was used to estimate the associations of $\beta_2$-GPI-Lp(a) with Lp(a), ox-Lp(a), degree of CAD, age, sex and plasma lipid levels.

tration of Ox-PL on the surface of Lp(a)\(^{19}\). In addition, $\beta_2$-GPI-Lp(a) complexes might also be taken up avidly by macrophages via anti-$\beta_2$-GPI autoantibody-mediated phagocytosis\(^{35}\), contributing to the development of atherosclerosis, and to be a risk factor for atherosclerosis.

The present study also showed that $\beta_2$-GPI-Lp(a) levels were positively associated with Lp(a), ox-Lp(a), and the extent of angiographically documented CAD, respectively. Multiple linear regression analysis showed that ox-Lp(a) and maximal stenosis accounted for 46.2% of the variation in the concentration of $\beta_2$-GPI-Lp(a). The relationships among Lp(a), ox-Lp(a) and Lp(a)-$\beta_2$-GPI imply that increased levels of Lp(a) of CAD patients result in more Lp(a) being able to be oxidized in vivo, subsequently forming $\beta_2$-GPI-Lp(a) complexes, which suggests that the plasma $\beta_2$-GPI-Lp(a) level will, to some extent, depend on ox-Lp(a) as well as Lp(a) levels. It has been
reported that $\beta_2$-GPI binds ox-LDL with higher affinity than native LDL due to increased negative charges on the surface of ox-LDL particles, resulting from the derivatization of lysine residues induced by lipid peroxidation of LDL. The oxidation pattern of Lp(a) is similar to that of LDL and the negative charges on the surface of ox-Lp(a) are also markedly increased as compared with corresponding native molecules. Thus, we supposed that $\beta_2$-GPI preferentially binds with ox-Lp(a) to native Lp(a), and increased levels of ox-Lp(a) in stable CAD and ACS patients result in forming more complexes of $\beta_2$-GPI-Lp(a).

What causes the closer association of $\beta_2$-GPI-Lp(a) levels with ACS than with stable CAD? The main probability is that $\beta_2$-GPI-Lp(a) concentrations depend on ox-Lp(a) as well as Lp(a) levels. In vitro studies have documented that macrophages and lymphocytes are capable of oxidizing lipoprotein. The culprit lesions of patients with ACS contain abundant macrophages and T lymphocytes. Under these circumstances, ox-Lp(a) in macrophage-derived foam cells may be enhanced within unstable plaques in association with the progression of plaque inflammation. On this basis, it could be hypothesized that ox-Lp(a) present within unstable plaques may be released into the bloodstream in patients with severe endothelial injuries, such as plaque erosion or rupture. In addition, the rise in plasma ox-Lp(a) may be a consequence of transient oxidative stress secondary to ischemia/reperfusion occurring during ACS, causing increased lipid peroxide. Our previous study also reported that ox-Lp(a) increased immediately after PCI. Thus, higher ox-Lp(a) could contribute to the marked increase in plasma $\beta_2$-GPI-Lp(a) in ACS. Another possibility is that Lp(a) might directly come from ruptured or permeable plaque contents. Although whether $\beta_2$-GPI-Lp(a) exists in atherosclerosis remains to be studied, it is possible that some ox-Lp(a) or Lp(a) particles might have combined with $\beta_2$-GPI.

The limitations of this study include the fact that the control subjects were not examined by coronary angiography to exclude potential CAD. In addition, we did not directly capture and analyze the embolized debris for $\beta_2$-GPI-Lp(a).

In conclusion, in addition to Lp(a) and ox-Lp(a), the presence of high $\beta_2$-GPI-Lp(a) is an important risk factor for the development and progression of atherosclerotic cardiovascular disease. These results should encourage further studies of the pathogenic role of $\beta_2$-GPI-Lp(a) complexes and the clinical value of detecting these complexes as a risk factor for the development of atherosclerosis.

Acknowledgements

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