β2-glycoprotein I deficiency: prevalence, genetic background and effects on plasma lipoprotein metabolism and hemostasis

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Abstract

β2-glycoprotein I (β2-GPI = apolipoprotein H) is an important autoantigen in patients with the antiphospholipid syndrome. It also plays a role in lipoprotein metabolism, such as anti-atherogenic property, triglyceride removal, and enhancement of lipoprotein lipase. Serum β2-GPI concentration of 812 apparently healthy Japanese individuals was measured by sandwich EIA. Two families with complete β2-GPI deficiency were identified. In one family, all affected had increased serum LDL-cholesterol levels or smaller particle sizes of LDL, while the other had no apparent abnormality in lipid metabolism. Individuals investigated had no history of thrombosis or overt abnormalities in hemostatic tests. A thymine corresponding to position 379 of the β2-GPI cDNA was deleted in every β2-GPI deficient individual. The incidence of this heterozygous deficiency determined by RFLP was 6.3% in Japanese and none in Caucasians. Heterozygotes had significantly lower concentrations of serum β2-GPI than did those without the mutation, yet no significantly different lipid profiles, such as total cholesterol, triglyceride, HDL-cholesterol, LDL-cholesterol, apoA-I, apoB and Lp(a), were observed. A low concentration of β2-GPI seemed not to be associated with apparent abnormality in lipoprotein metabolism. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: β2-glycoprotein I; Deficiency; Lipoprotein metabolism; Hemostasis; Atherosclerosis; Antiphospholipid syndrome

1. Introduction

β2-glycoprotein I (β2-GPI = apolipoprotein H) plays a central role in the pathogenesis of the antiphospholipid syndrome (APS) [1]. ‘Anticardiolipin’ antibodies found in many patients with APS bind to a complex of cardiolipin and β2-GPI [2–4]. Recent studies indicate that those anticardiolipin antibodies (aCLs) are directed to β2-GPI itself [5,6].

β2-GPI is a plasma apolipoprotein with an approximate molecular weight of 50 kD. Physiological functions of β2-GPI have not been clarified, but β2-GPI does contribute to triglyceride clearance from rat plasma [7], and β2-GPI binds oxidized lipoproteins and inhibits the uptake and proteolytic degradation of oxidized low-density lipoprotein by macrophages [8]. Thus, β2-GPI may possibly function as an anti-atherogenic factor in vivo.

β2-GPI exhibits anticoagulatory properties by inhibiting the phospholipid dependent coagulation pathway [9], although procoagulant activities by reduction of protein C activity have been suggested [10]. Protein C activity is further inhibited by monoclonal aCLs established from APS patients [11].

β2-GPI consists of five repeating domains designated domain I–V. Unlike other domains, domain V contains two additional cysteines and a long C-terminal tail, and is reported to include the binding site to negatively
charged surfaces. Therefore, domain V is considered to be essential for β₂-GPI to exert physiological functions [12]. On the other hand, domain IV is considered to provide the binding site for aCL in APS patients and to play a pathologically important role [13]. The hemodynamics of β₂-GPI are not well understood. We established a sandwich enzyme immunoassay (EIA) system to measure β₂-GPI concentration, in order to determine serum β₂-GPI concentrations in normals and those with various disorders.

In studies on a normal Japanese population in the Hokkaido area of Japan, we identified two subjects in whom serum β₂-GPI was undetectable. After identifying the mutation of the β₂-GPI gene responsible for the low β₂-GPI concentrations, we investigated the frequency of this genetic polymorphism in a fairly large Japanese population. We also studied possible changes in profiles and chemical compositions of plasma lipoproteins in persons with homozygous or heterozygous β₂-GPI deficiencies. Careful attention was directed to the quality of the sample group in order to avoid effects on plasma lipoprotein metabolism of other factors, such as obesity, diabetes, hypertension, alcohol consumption and use of tobacco. We found that this polymorphism in the β₂-GPI gene is fairly common in the Japanese population. The consequences of β₂-GPI deficiency are discussed.

2. Methods

2.1. Subjects and sample collection

2.1.1. Study 1

Subjectively healthy Japanese individuals (n = 812), living in the Hokkaido area of Japan, were studied with special attention directed to serum β₂-GPI concentrations. Written informed consent was obtained from all the individuals included in this study. Fasting peripheral blood was obtained and the sera were stored at −80°C until use. Individuals with undetectable serum β₂-GPI concentrations, and their family members were subjected to genomic DNA analysis and also studies on lipoprotein metabolism and hemostasis were done.

2.1.2. Study 2

In 222 subjects (designated as the reference group; 85 men and 137 women, 21–72 years old) of the above 812, we studied the incidence of the identified mutation and the effect of β₂-GPI deficiency on lipoprotein metabolism. To avoid bias due to possible effects of the factors other than β₂-GPI, only subjects fulfilling the following criteria were included: (i) normotensive (systolic blood pressure below 160 mmHg, diastolic blood pressure below 95 mmHg); (ii) body mass index within 22 ± 10%; (iii) fasting blood glucose under 110 mg/dl; (iv) hemoglobin level exceeding 13 g/dl in men, over 11 g/dl in women; (v) non-smokers; and (vi) non-habitual drinkers of alcohol. Subjects found deficient in cholesteryl ester transfer protein (CETP), in a previously reported PCR/RFLP analyses, were excluded from the study group, because CETP deficiency is relatively common in the Japanese, and affects lipid metabolism [14].

β₂-GPI gene polymorphism identified in Study 1 was examined in the reference group, using genomic DNA from peripheral blood cells. Sera obtained from the reference group were stored at −80°C until the measurements of lipids and apolipoproteins. DNA samples from 131 Caucasian healthy individuals were obtained for comparison.

2.2. Sandwich EIA

Cof 20 antibody and Cof 23 antibody are mouse monoclonal anti-human β₂-GPI antibodies specific to domain III and IV of human β₂-GPI, respectively [13]. Wells of Sumilon S plates (Sumitomo Bakelite, Tokyo, Japan) were coated with 50 μl/well of 5 μg/ml Cof 20 antibody diluted with phosphate buffered saline (PBS) and incubated overnight at 4°C. After three washes with PBS, wells were blocked with 50 μl/well of 1% bovine serum albumin (BSA) diluted with PBS for 1 h. Fifty microlitres/well of serum samples diluted 1:1000 in PBS containing 0.5% Tween20 (PBS-tween) were distributed to each well and the plates incubated at room temperature for 1 h. After three washes with PBS-tween, wells were incubated with 50 μl/well of biotinylated Cof 23 antibody (1 μg/ml). Following incubation for 1 h and three washes with PBS-tween, 50 μl/well of AP-Streptavidin (Zymed, South San Francisco, CA) diluted to 1:5000 in PBS-tween was added. After a 15 min incubation at room temperature and four washes with PBS-tween, phosphate substrate tablets (SIGMA, St. Louis, MO) dissolved with 1 M diethanolamine buffer was added and optical density (OD) at 405 nm was measured. Serial dilutions of β₂-GPI were included in each assay to generate standard curves. Either serum or plasma samples yielded similar concentrations (Data not shown).

2.3. Sequence analysis of the β₂-GPI gene

Genomic DNA for human β₂-GPI consists of eight exons [15]. PCR primers for each exon described by Sanghera et al. [15] were used to amplify the β₂-GPI gene in individual samples. Genomic DNA was isolated from peripheral blood using DnaQuick kits (Dainippon Seiyaku, Osaka, Japan), and were subjected to PCR for each exon of β₂-GPI. AmpliTaq Gold (Perkin-Elmer, Norwalk, CT) and its standard buffer were used in all reactions. PCR conditions were as follows; 10 μl of
reaction containing 50 ng DNA, 5 pmol of each primer, 1 µl of 10 × PCR buffer II, 0.8 µl of dNTP mix (2 µM of each dNTP), 0.6 µl of MgCl₂ (25mM), and 0.08 µl of ampliTaq Gold (5 U/µl) was subjected to initial denaturing at 94°C for 8 min, followed by 35 cycles of denaturing at 94°C for 0.5–1 min, annealing step at 56–8°C for 0.5–1.5 min, and extension step at 72°C for 0.5–2 min, and an additional extension step at 72°C for 6 min. Amplified products were ligated into pCR II vectors (Invitrogen, San Diego, CA). Vectors were purified and their inserts were sequenced using ABI PRISM dye terminator cycle sequencing kits (Perkin-Elmer).

2.4. Restriction fragment length polymorphisms

Exon 4 of the β₂-GPI gene was amplified by PCR using genomic DNA samples. Five microlitres of PCR products were digested with 3 units of ApaL-1 (New England Biolabs, Revely, MA) at 37°C for 8 h and applied to a 4% agarose gel. ApaL-1 will cut the amplified products from the normal allele to two fragments of 109 and 99 bp. Products amplified from genes with the mutation studied in this paper will remain undigested. An electrophoretic pattern with 109 and 99 bp bands corresponds to the normal allele, a pattern with 208 bp band to heterozygous β₂-GPI deficiency, and that with 109, 99 and 208 bp bands to homozygous β₂-GPI deficiency.

2.5. Serum lipid analysis

Total cholesterol (TC) and triglyceride (TG) were determined enzymatically, using commercial kits (Kyowa Medex, Tokyo, Japan). High density lipoprotein-cholesterol (HDL-C) was measured in a homogenous assay (Daichi Pure Chemicals, Tokyo, Japan). Serum LDL-C was calculated by the Friedewald’s formula [16]. Concentrations of apolipoproteins (apo A-I, A-II, B, C-II, C-III, and E) in sera were determined in [16]. Concentrations of very low-density lipoproteins (VLDL), LDL, and HDL were isolated and concentrated enzymatically, as described above. Protein concentrations were determined by the method of Lowry et al. [18] as modified by Markwell et al.[19], using BSA as standard.

2.7. Lipoprotein electrophoresis

Agarose gel electrophoresis was carried out using Universal Gel (Helena Laboratories, Beaumont, TX) in 50 mmol/l barbital buffer (pH 8.6), and serum lipoproteins were stained with Fat Red B. Native polyacrylamide gel electrophoresis, using Lipophor System (Quantimetrix Co., Hawthorne, CA) was done in order to evaluate the sizes of lipoprotein particles.

2.8. LDL receptor assay

LDL receptor activity was determined by measuring the uptake of fluorescence-labelled LDL by peripheral blood mononuclear cells, using a flow cytometer and 1, 1'-Diocadecyl-3, 3', 3'-tetramethylindocarbocyanine perchlorate, DiI, as the label [20].

2.9. Hemostatic analyses

The following hemostatic variables in citrated plasma of homozygous β₂-GPI deficient individuals were measured; prothrombin time, activated partial thromboplastin time, plasma fibrinogen, antithrombin III activity, plasma levels of prothrombin fragment 1 + 2 (Enzygnost F1 + 2 micro, DADE Behring, Germany), thrombin–antithrombin complex (TAT test Kokusai F, Kokusai Shiyaku, Japan), plasminogen–plasmin inhibitor complex (PIC test Kokusai F, Kokusai Shiyaku), D-dimer antigen (LPIA200 D-dimer, Diatron), protein C activity (Protac, Chromogenix, Mölndal, Sweden), protein S antigen (Asserachrom Total Protein S, Diagnostica Stago, Asnieres, France) and free protein S antigen (Asserachrom Free Protein S, Diagnostica Stago).
3. Results

3.1. Serum $\beta_2$-GPI concentrations

Serum $\beta_2$-GPI concentrations in the 812 subjectively healthy volunteers ranged from less than the minimum detection limit (5 μg/ml) to 625 μg/ml (236 ± 104 μg/ml, mean ± SD). The histogram was mildly skewed to the left (Fig. 1). In two unrelated subjects, $\beta_2$-GPI was undetectable.

3.2. Family studies

Pedigree maps of the two subjects with undetectable $\beta_2$-GPI levels are shown in Fig. 2. In one family (Family A), the proband (A-IV-1) was a 36-year-old subjectively healthy female. Western blotting using Cof 20 and 21, and immunoelectrophoresis, using polyclonal anti-human serum antibodies, did not show a band corresponding to $\beta_2$-GPI in the proband (data not shown). In the other family (Family B), the proband (B-IV-1) was a 34-year-old woman, apparently healthy except for the presence of mild iron deficiency anemia.

The sequence analysis of genomic DNA from the two probands revealed that a thymine corresponding to position 379 of $\beta_2$-GPI cDNA was deleted in both probands (Fig. 3). This position is present on exon 4 and codes the domain II of the $\beta_2$-GPI protein [21]. The concomitant frame shift will make the gene code for amino acid sequences unrelated to $\beta_2$-GPI, beyond this position. A stop codon appears at the beginning of exon 6, which originally codes for the domain IV of $\beta_2$-GPI (Fig. 4). Other exons in their genes showed sequences identical to those previously reported [21].

DNA analysis revealed that A-IV-2 was also homozygous for the same mutation. In addition, A-III-2 and B-III-2 were found to be heterozygotes. Serum $\beta_2$-GPI concentrations for A-III-2 and B-III-2 were 36 and 55 μg/ml, respectively. In Family A, two siblings were without symptoms although A-III-2 had diabetes. A-III-1 died of a stroke at age 61. He had had hypertension, which was well controlled with drugs. Several members of this family had died of stroke or myocardial infarction. On the other hand, none of the three persons we could investigate had a history of thrombosis. In Family B, B-III-2 had hypertension and was on medication. B-III-1 had died of unknown causes at age 42.

Hemostatic variables were measured in all of the homozygous $\beta_2$-GPI deficient individuals of Family A and B. Prothrombin time, activated partial thromboplastin time, plasma fibrinogen, and antithrombin III activity were normal. Thrombin generation and fibrinolytic turnover in vivo were evaluated by measuring plasma levels of prothrombin fragment 1 + 2,
thrombin–antithrombin complex, plasminogen–plasmin inhibitor complex and D-dimer antigen. All of those parameters were within normal ranges, suggesting that \( \beta_2 \)-GPI deficiency is not associated with subclinical thrombotic tendency. In none of these persons was there protein C or protein S deficiency.

As shown in Table 1, A-IV-2 and A-III-2 had elevated TC, LDL-C, and apo B levels, indicating an increase of LDL in plasma. Apo C-II, apo C-III, and apo E levels were also moderately elevated, indicating an increase of VLDL. In A-IV-1, similar but mild changes were observed. In Family B, no definite changes in lipoprotein variables were observed, except for mild elevation in apo B in B-III-2. Lp(a) and CETP levels were normal in all subjects in Family A and B. BMI was higher than the consensus value in A-IV-1, A-III-2, and B-III-2. The LDL receptor activity of peripheral blood mononuclear cells determined in A-IV-1 was 159% (normal > 80%).

In native PAGE (Fig. 5), LDL particles were polydisperse and smaller in A-III-2 and A-IV-1, which was similar to the pattern in a patient with type II b hyperlipidemia without \( \beta_2 \)-GPI deficiency, studied for comparison. Slightly polydisperse LDL particles were observed in B-III-2.

In agarose gel electrophoresis (Fig. 6), relatively dense \( \beta \) and pre \( \beta \) bands were observed in each member of Family A, while no definite change was observed in Family B.

Chemical compositions of isolated lipoproteins are shown in Table 2. No remarkable change was found in VLDL in either family. Slightly lower phospholipid contents in LDL were observed in the both families. HDL had a higher triglyceride content in the members of Family A, while a higher cholesterol content and a lower protein content were found in two members of Family B.

### 3.3. \( \beta_2 \)-GPI deficient allele in healthy individuals; frequency and effects on serum \( \beta_2 \)-GPI concentration and lipid metabolism

Incidence of heterozygous deficiency as analyzed by RFLP was 6.3% (14/222) in the reference group. One hundred and thirty one Caucasian healthy individuals did not have this mutation of the \( \beta_2 \)-GPI gene. Persons with heterozygous deficiency had significantly lower concentrations of serum \( \beta_2 \)-GPI than did controls (normals; 243 ± 99, heterozygotes; 86 ± 31, \( P \) value calculated by Mann–Whitney’s \( U \) test; < 0.0001) (Table 3). No significant differences were observed between normal persons and heterozygotes, in lipoprotein variables, including fasting TC, TG, HDL-C, LDL-C, apo A-I, apo B and Lp(a) (Table 3). No significant relationship
<table>
<thead>
<tr>
<th></th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
<th>ApoAI (mg/dl)</th>
<th>ApoAII (mg/dl)</th>
<th>ApoB (mg/dl)</th>
<th>ApoCIII (mg/dl)</th>
<th>ApoE (mg/dl)</th>
<th>Lp(a) (mg/dl)</th>
<th>CETP (mg/dl)</th>
<th>BMI (m/kg²)</th>
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<tr>
<td>A-III-2 (hereto)</td>
<td>310</td>
<td>183</td>
<td>40</td>
<td>233</td>
<td>111</td>
<td>28.0</td>
<td>195</td>
<td>12.5</td>
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<td>A-IV-1 (homo)</td>
<td>232</td>
<td>157</td>
<td>35</td>
<td>166</td>
<td>91</td>
<td>27.6</td>
<td>120</td>
<td>6.6</td>
<td>10.7</td>
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<td>302</td>
<td>143</td>
<td>44</td>
<td>229</td>
<td>117</td>
<td>36.0</td>
<td>174</td>
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<td>12.3</td>
<td>7.0</td>
<td>2.5</td>
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<td>B-III-2 (hetero)</td>
<td>245</td>
<td>64</td>
<td>69</td>
<td>163</td>
<td>153</td>
<td>33.5</td>
<td>123</td>
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<td>8.2</td>
<td>4.5</td>
<td>24.6</td>
<td>27.6</td>
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<tr>
<td>B-IV-1 (homo)</td>
<td>161</td>
<td>45</td>
<td>67</td>
<td>148</td>
<td>148</td>
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<td>65</td>
<td>1.2</td>
<td>6.7</td>
<td>4.8</td>
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<td>Reference interval</td>
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<td>34–94</td>
<td>70–177</td>
<td>110–160</td>
<td>25–36</td>
<td>68–100</td>
<td>1.9–4.5</td>
<td>5.5–10.5</td>
<td>3.1–4.9</td>
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<td>Consensus value</td>
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<td>&lt; 150</td>
<td>&gt; 40</td>
<td>&lt; 140</td>
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<td>&lt; 140</td>
<td>&lt; 140</td>
<td>&lt; 140</td>
<td>&lt; 140</td>
<td>&lt; 140</td>
</tr>
</tbody>
</table>

TC, total cholesterol; TG, triglyceride; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; apo, apolipoprotein; Lp(a), lipoprotein (a); CETP, cholesteryl ester transfer protein; BMI, body mass index. Numbers written in italics are out of reference intervals. Reference interval 95% reliable interval (non-parametric); consensus values are those recommended by Japan Society for the Study of Obesity.
4. Discussion

The pathogenic role of aCLs, especially anti β₂-GPI antibodies, has been extensively studied, yet the exact mechanisms of thrombosis in APS have not been clearly defined. One possible explanation is the acceleration of atherosclerosis by aCL [8,22] and superimposed thrombotic events, and another is the perturbation in hemostasis by aCL, such as inhibition of protein C system [11] or of the anticoagulative properties of β₂-GPI itself.

Similarly, the role of β₂-GPI in lipid metabolism is also obscure. Around 30% of serum β₂-GPI resides in the lipoprotein fraction. Thus, this protein is also known as apolipoprotein H and is considered to have some roles in lipid metabolism [23]. Nakaya et al. [24] reported that β₂-GPI increased the enzymatic activity of lipoprotein lipase in the presence of apoC-II. Wurm et al. [7] reported that administration of β₂-GPI enhanced the removal of triglyceride from blood in rats. Köchel et al. [25] reported that Lp(a) binds to β₂-GPI and that this reaction could represent a possible route by which Lp(a) is cleared from plasma. β₂-GPI was found to bind oxidized LDL, and to prevent endocytosis of oxidized LDL by macrophages via scavenger receptors [8]. If this in vitro finding holds true in vivo, β₂-GPI would have anti-atherogenic properties by preventing the increase in foam cells. Furthermore, shortage or absence of β₂-GPI in sera may enhance the progression of atherosclerosis.

β₂-GPI shows anticoagulant properties by inhibiting contact activation of phospholipid-dependent coagulation pathway [9], platelet prothrombinase activity [26] and ADP-induced platelet aggregation [27] in vitro. Shiozaki et al. [28] reported that β₂-GPI inhibits the intrinsic blood coagulation pathway, when added in excess to β₂-GPI depleted plasma. On the other hand, Mori et al. [10] showed that β₂-GPI can inhibit the anticoagulant activity of activated protein C, which is enhanced by aCL [11]. Thus, it is currently considered that β₂-GPI has both anticoagulant and procoagulant properties.

Although the in vitro studies described above suggest that β₂-GPI may have a role in lipid metabolism, and that it has some effect on coagulation, clinical studies showed that β₂-GPI deficiency is not a risk factor for thrombosis [29] or bleeding tendency and does not result in any significant perturbation of lipoprotein metabolism [30]. However, in one family study [29], some members had thrombotic diseases following stressful events such as trauma or surgery, and in another study [30], two of five homozygote β₂-GPI deficient persons had a history of myocardial infarction. We speculate that homozygous β₂-GPI deficiency may be an additional, if not a dependent, risk factor for atherosclerosis or thrombosis. Therefore, it is important to investigate the relationship between β₂-GPI concentration and lipoprotein metabolism, to compare lipid profiles between β₂-GPI deficient individuals and normals, and also to study effects of β₂-GPI deficiency on hemostasis.

The lipid profiles of the three homozygous β₂-GPI deficient persons were not consistent. The two siblings in Family A (Fig. 2, A-IV-1 and -2) had an abnormal lipid metabolism with increased smaller LDL, which is more atherogenic than is larger LDL [31] and suggests the existence of excessive lipid peroxidation. However, the β₂-GPI deficient person in Family B had no such abnormality. Hence it is possible that the abnormal
lipoprotein profiles found in Family A was not the direct result of β2-GPI deficiency. Smaller LDL can be commonly observed in persons with elevated VLDL as shown in Fig. 5, and both of the A-IV-1 and 2 had elevated VLDL components such as apo B, CII, CIII, and E (Table 1) and electrophoretic patterns similar to that for type IIb hyperlipidemia (Fig. 6). It is possible that other factors in Family A, such as diabetes, obesity, and familial combined hyperlipidemia, had affected their lipid profiles. As shown in Table 2, in the β2-GPI deficient subjects, percentages of phospholipid included in LDL is somewhat smaller than normals, although the consequences of these small changes are unclear.

Sequencial analysis of genomic DNA from our β2-GPI deficient subjects showed that a frame shift would occur in position 379 of the β2-GPI cDNA, corresponding to domain II of the β2-GPI protein. Therefore, domain I and a small part of domain II of β2-GPI may be intact, but it is unclear whether this protein is secreted or is not produced at all, because we do not possess antibody specific to domain I of β2-GPI. Heterozygotes for this β2-GPI gene polymorphism are relatively common (6.3%) in the Japanese. On the other hand, this gene polymorphism is quite uncommon in Caucasians. As the allele frequency of β2-GPI deficiency, determined by serum concentration of β2-GPI in Caucasians is reported to be 4–6% [32], the mutation in these β2-GPI deficiencies may be different from that of the Japanese, or there may be some other reasons.

Several β2-GPI gene polymorphisms, such as Ser 88 Asn, Val 247 Leu, Ser 306 Gly and Trp 316 Ser, and corresponding protein structural polymorphisms are reported [15,21,33,34]. Mutations of Gly 306 and Ser 316 in domain V render β2-GPI unable to bind negatively charged phosphatidylserine [35]. Val 247 was considered to be important in the formation of β2-GPI antigenicity in Caucasian primary APS patients [36]. In particular, codon 316 polymorphism has recently reported to be a determinant of plasma β2-GPI concentration [34,37]. Patients homozygous for this missense mutation had significantly lower serum β2-GPI concentrations, but still well above the levels seen in individuals with heterozygous β2-GPI deficiency observed in the current study.

In our study, individuals with heterozygous β2-GPI deficiency showed significantly lower concentrations of serum β2-GPI, but no significant influence on lipid metabolism was found, at least in the fasting state. These findings imply that either a low concentration of β2-GPI is sufficient to exert its function in regard to lipoprotein metabolism, or β2-GPI have no significant roles in lipoprotein metabolism.

Although it is considered that β2-GPI deficiency is not a major risk factor of thrombosis, taking into account previous reports and our results indicating that routine coagulation assays were normal and no thrombotic episodes were found in the homozygous β2-GPI deficient persons, it cannot be determined if β2-GPI deficiency is an additional risk factor of thrombosis. Risk factors such as tobacco use, obesity, a cholesterol-rich diet or impaired glucose tolerance may render β2-GPI deficient individuals more susceptible to atherosclerosis, or even to thrombosis. Further investigations are needed to determine the hemostasis of β2-GPI deficient plasma, when stimulated by clotting triggers. A long term analysis and a larger population will be necessary to clarify how β2-GPI deficiency af-

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Component</th>
<th>A-III-2</th>
<th>A-IV-1</th>
<th>A-IV-2</th>
<th>B-III-2</th>
<th>B-IV-1</th>
<th>Normal (n = 8, mean ± SD)</th>
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<td>Cholesterol</td>
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<td>15.3</td>
<td>10.2</td>
<td>15.0</td>
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<td>14.0 ± 2.2</td>
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<td></td>
<td>Free cholesterol</td>
<td>5.5</td>
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<td>5.6</td>
<td>nd</td>
<td>6.3 ± 1.2</td>
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<td>Triglyceride</td>
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<td>49.3</td>
<td>54.9</td>
<td>54.3</td>
<td>nd</td>
<td>47.6 ± 6.8</td>
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<td>Phospholipid</td>
<td>18.1</td>
<td>18.4</td>
<td>21.8</td>
<td>16.1</td>
<td>nd</td>
<td>20.8 ± 2.3</td>
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<td>Protein</td>
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<td>8.5</td>
<td>9.0</td>
<td>nd</td>
<td>11.4 ± 1.7</td>
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<tr>
<td>LDL</td>
<td>Cholesteryl ester</td>
<td>41.1</td>
<td>41.4</td>
<td>43.6</td>
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<td>7.6</td>
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<td>7.5</td>
<td>7.4</td>
<td>8.4 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>Phospholipid</td>
<td>20.2</td>
<td>21.8</td>
<td>19.8</td>
<td>21.4</td>
<td>20.5</td>
<td>22.5 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>Protein</td>
<td>19.4</td>
<td>19.5</td>
<td>19.6</td>
<td>18.8</td>
<td>22.7</td>
<td>21.9 ± 1.3</td>
</tr>
<tr>
<td>HDL</td>
<td>Cholesteryl ester</td>
<td>18.1</td>
<td>17.3</td>
<td>18.5</td>
<td>25.1</td>
<td>21.6</td>
<td>17.6 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>Free cholesterol</td>
<td>3.5</td>
<td>2.1</td>
<td>2.4</td>
<td>3.5</td>
<td>3.6</td>
<td>2.4 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Triglyceride</td>
<td>5.2</td>
<td>4.9</td>
<td>6.0</td>
<td>2.8</td>
<td>3.1</td>
<td>2.3 ± 0.9</td>
</tr>
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<td>24.6</td>
<td>25.5</td>
<td>21.5</td>
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<td>26.0</td>
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<tr>
<td></td>
<td>Protein</td>
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<td>50.2</td>
<td>51.6</td>
<td>43.8</td>
<td>45.8</td>
<td>52.4 ± 1.8</td>
</tr>
</tbody>
</table>

* VLM, very low density lipoprotein; LDL, low density lipoprotein; HM, high density lipoprotein; nd, not determined for too low concentrations. Cholesteryl ester concentrations were calculated by multiplying the esterified cholesterol concentrations, obtained by subtracting free cholesterol from total cholesterol by 1.72. Numbers written in italics are out of normal values (mean ± 2 SD).
fects lipid metabolism, and to determine whether β₂-GPI deficiency is a risk factor for atherosclerosis or thrombosis.

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References


