

Beta 2 glycoprotein I Valine247Leucine polymorphism in patients with antiphospholipid syndrome

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ABSTRACT

Aim: Beta 2 Glycoprotein I (β 2-GP I) takes part in the pathogenesis of antiphospholipid syndrome (APS). Valine247Leucine (Val247Leu) gene polymorphism of β 2-GP I might affect the binding/production of anti- β 2-GP I antibodies. Multiple studies are showing different frequencies of this polymorphism in various ethnic backgrounds; we aimed to determine the frequency and clinical importance of Val247Leu gene polymorphism of β 2-GP I in patients with APS and healthy.

Methods: Eighty-three patients with APS [68 primary APS, 15 APS with systemic lupus erythematosus (SLE)] and 63 healthy individuals were included. B2-GP I Val247Leu polymorphism was determined by quantitative real time polymerase chain reaction and melting curve analysis. The presence of anti- β 2-GP I antibodies was detected by ELISA in the patient group.

Results: Allele and genotype frequencies were similar between patients and healthy controls ($p=0,307$). V allele and VV genotype frequencies were significantly higher in primary APS patients with thrombocytopenia ($p=0.040$). There was no significant difference between β 2-GP I Val247Leu gene polymorphism and the anti- β 2-GP IgM and IgG antibody levels in the patient group ($p=0.631$ and $p=0.077$, respectively)

Conclusion: This is the first study investigating the β 2-GP I Val247Leu gene polymorphism in the Turkish population. The frequencies of Val247Leu gene polymorphism of β 2-GP I were not different between patients with APS and healthy individuals in line with the other studies in Caucasian populations. Significantly high levels of V allele and VV genotype frequencies in primary APS patients could offer further insight to into the pathogenesis of thrombocytopenia in APS.

Key words: Antiphospholipid syndrome, beta 2 glycoprotein I, gene polymorphism, thrombocytopenia.

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Introduction

Antiphospholipid syndrome (APS) is characterized by with arterial/venous thrombosis and or pregnancy morbidity in the presence of persistently elevated antiphospholipid antibodies (aPL) [1, 2].

Initially, aPL were assumed to interact directly with phospholipids; but in further studies, their true target was proved to be beta 2 glycoprotein I (β 2-GP I), a phospholipid binding plasma protein [3, 4, 5] [3-5].

B2-GP I is a 43-kD protein that circulates at a concentration of 150-300 μ g/mL. It consists of 326 amino acids (aa) arranged in five short consensus repeat domains (SCR) [6, 7]. The crystal structure of the molecule revealed a hook-like appearance. The first four domains are regular SCR domains with the same aa sequences; the fifth domain, which deviates from the usual alignment, forms the binding site for anionic phospholipids [8]. After binding of β 2-GP I to a negatively charged surface, cryptic epitope is exposed and aPL bind to β 2-GP I [9]. The β 2-GP I gene is located in chromosome 17 q23-24 and is composed of 8 exons, with variations among individuals of the same ethnic group [10]. The polymorphism at position 247 involves valine (Val/V) or leucine (Leu/L) aa between the phospholipid binding site of the fifth domain and cryptic epitope of the fourth domain. The importance of antigen polymorphism in the production of autoantibodies has been extensively studied; aa substitution of antigens can lead to differences in antigenic epitopes of a given protein. The Val247Leu gene polymorphism of β 2-GP I in the fifth domain may affect binding or production of anti- β 2-GP I antibodies that could play a role in pathogenesis of APS [11]. There are various studies investigating the distribution of Val247Leu polymorphism and its association with clinical manifestations and anti- β 2-GP I antibody titer among different ethnic groups. Studies in Asian [11, 12] and South American [13-15] populations have identified clinical and serological associations between APS and β 2-GP I gene polymorphism but in studies with Caucasian populations this

information was not confirmed [16-19].

In this cross-sectional study, we investigated the presence of the polymorphism at position 247 of the β 2-GP I gene in patients with APS and healthy individuals. Association with clinical manifestations and Anti- β 2-GP I antibody levels were also evaluated.

Materials and methods

We studied 83 patients with APS [68 primary APS and 15 with systemic lupus erythematosus (SLE)] followed up by Istanbul University Hematology and Rheumatology departments. All patients satisfied the classification criteria for APS [2] and the patients with SLE satisfied the American College of Rheumatology criteria for SLE [20].

Patients and healthy individuals provided written informed consent. The study was approved by Istanbul University Ethics Committee for Clinical Studies (2008/2157) and performed per the Declaration of Helsinki. Sixty-three healthy individuals with no history of autoimmune or thrombotic disease were included into the study.

B2-GP I Val247Leu polymorphism

Genomic DNA was extracted from peripheral white blood cells using DNA isolation kit as

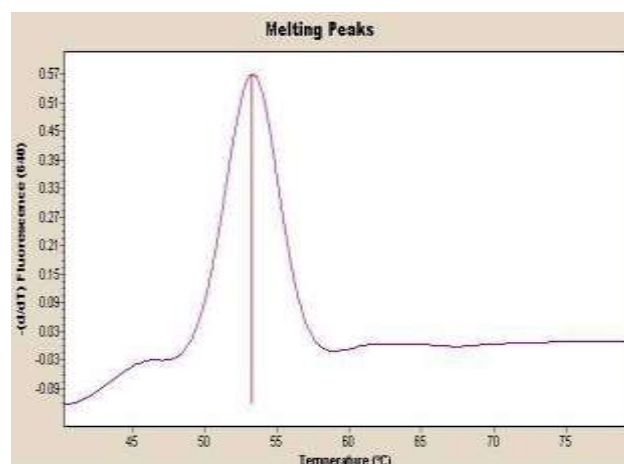


Figure 1. Quantitative real time PCR image; VV, 51 °C. PCR: Polymerase chain reaction, V: Valine.

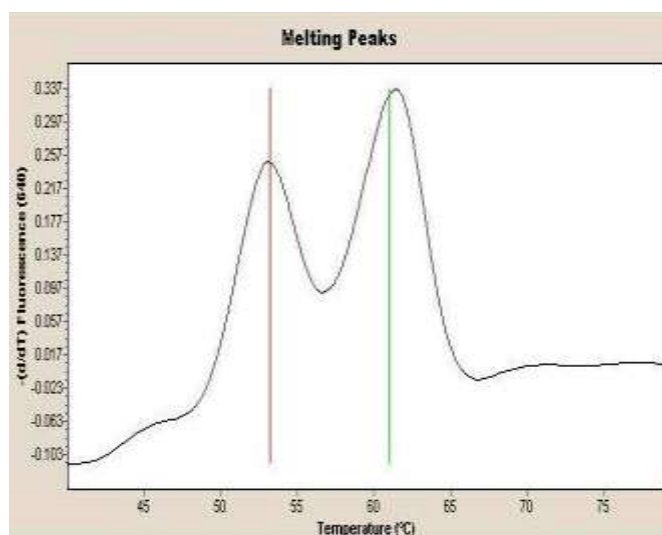


Figure 2. Quantitative real time PCR image VL, 51°C and 63 °C. PCR: Polymerase chain reaction, V: Valine, L: Leucine.

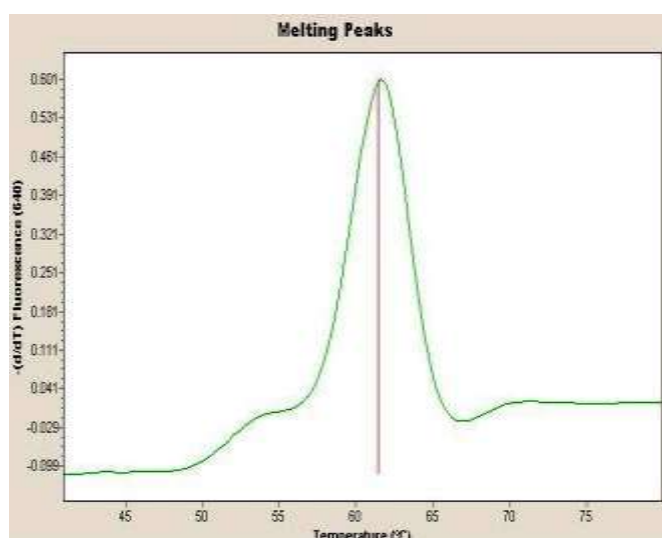


Figure 3. Quantitative real time PCR image LL, 63 °C. PCR: Polymerase chain reaction, L: Leucine.

described by the manufacturer (Roche Diagnostics, Mannheim, Germany). Specific primers were designed with Primer Express 3.0 software (Applied Bio-systems, Foster City, CA, USA). Primers and probes, template DNA, MgCl₂ were used for Quantitative Real Time Polymerase Chain Reaction (Q-RT PCR) (Light® Cyclor, Roche). PCR conditions were;

5 minutes at 94°C, 30 seconds (sec) at 94°C, 30 sec at 57°C, 20 sec at 72°C (50 cycles), 30 sec at 94°C, 30 sec at 57°C, 20 sec at 72°C (one cycle). Genotypes were determined with melting curve analysis (Figure 1-3).

Anti-β2-GP I Antibodies

Anti-β2-GP I IgM and IgG antibodies were determined with Quanta Lite™ β2-GP I IgM and IgG ELISA kit (INOVA Diagnostics, San Diego, CA, USA) as described by the manufacturer. A 1:100 dilution of each patient sample was prepared by adding 5 μL serum to 500 μL HRP sample diluent. 100 μL of diluted patient samples were added to the wells and incubated for 30 minutes at room temperature. Each well was aspirated and 250 μL HRP wash buffer was added to the wells and aspirated twice. After the last aspiration step 100 μL HRP IgM or IgG conjugate was added to the wells and incubated for 30 minutes at room temperature. Each well was aspirated and 250 μL HRP wash buffer was added to the wells and aspirated twice. After last aspiration step 100 μL TMB chromogen was added to the wells and incubated in the dark for 30 minutes at room temperature. The reaction was stopped using 100 μL HRP stop solution for each well. Optical density absorbance of each well was read at 450 nm and results were evaluated.

Statistical analysis

Data were analyzed with Number Cruncher Statistical System 2007 and PASS 2008 Statistical Software (Utah, USA). Qualitative variables were analyzed with the chi-square test and Fisher's exact test. Descriptive statistics for quantitative variables were expressed as mean ± standard deviation (SD). Differences between two variables were analyzed with Student's t test if normally distributed; if not Mann Whitney U test was used. Kruskal Wallis test

was used to analyze the difference between three group groups with normal distribution. $p < 0.05$ was considered statistically significant.

Results

The study group included 83 patients with APS [68 primary APS and 15 with systemic lupus erythematosus (SLE)]. The median age of the patients was 38 years (18-68); the female to male ratio was 61: 22. Clinical manifestations were as follows: 38 (45.8 %) had a history of arterial thrombosis, 26 (31.3%) had venous thrombosis, 8 (9.6%) had both arterial and venous thrombosis; 15 (18%) had thrombocytopenia; 26 out of 61 female patients had (42.6%) recurrent pregnancy losses (some patients had more than one manifestation).

The healthy control group included 63 individuals with a median age of 41 years (18-68) and the female to male ratio of 33: 30.

Allele and genotype frequencies of $\beta 2$ -GP I Val247Leu polymorphism:

We evaluated allele and genotype frequencies of $\beta 2$ -GP I Val247Leu gene polymorphism in 83 APS patients and 63 healthy individuals. Allele or genotype frequencies were not significantly different between APS patients and healthy individuals ($p > 0.05$) (Table 1-2).

VV genotype was detected in 13 (15.7%) patients and 8 (12.7%) healthy individuals. VL genotype was detected in 30 (36.1%) patients and 30 (47.6%) healthy individuals; LL genotype was detected in 40 (48.2%) patients and 25 (39.7%) healthy individuals. V allele frequency was 33.73% in APS group and 36.5% in healthy individuals; L allele frequency was 66.27% in APS group and 63.5% in healthy individuals. This allele and genotype frequencies did not differ between primary APS patients and APS patients with SLE.

Table 1. Genotype expression in each group.

Genotype	APS (n=83)	Healthy Individuals (n=63)	Total	p
	n (%)	n (%)	n (%)	
VV	13 (15.7)	8 (12.7)	21 (14.4)	0,37
VL	30 (36.1)	30 (47.6)	60 (41.1)	
LL	40 (8.2)	25 (39.7)	65 (44.5)	

V: Valine, L: Leucine, APS: Antiphospholipid syndrome. Chi-square test.

Table 2. Allele frequencies in the each group.

Allel	APS (n=83)	Healthy Individuals (n=63)	p > 0.05
	(%)	(%)	
V	33.73%	36.5%	
L	66.27%	63.5%	

V: Valine, L: Leucine, APS: Antiphospholipid syndrome. Chi-square test.

B2-GP I Val247Leu gene polymorphism and anti- $\beta 2$ -GP I antibody levels

In our study, we also evaluated the relationship between $\beta 2$ -GP I Val247Leu polymorphism and anti- $\beta 2$ -GP I antibody levels in patients with APS. Anti- $\beta 2$ -GP I antibody levels were measured in 66 APS patients. Not all of the patients who had genotype testing had anti- $\beta 2$ -GP I antibody positivity.

Even though there was no significant difference between $\beta 2$ -GP I Val247Leu polymorphism and the anti- $\beta 2$ -GP IgM or IgG antibody levels in patient group ($p = 0.631$ and $p = 0.077$, respectively), high levels of anti- $\beta 2$ -GP IgG were remarkable in LL genotype (Table 3).

Table 3. Anti-β2-GP I antibody levels in the each genotype of the APS patients.

Parameters	Genotype			p
	VV	VL	LL	
	Mean ± SD (Median)	Mean ± SD (Median)	Mean ±SD (Median)	
Anti-β2-GP I IgM	13.54±64.93 (-19.1)	10.43±56.77 (-12.36)	45.05±62.46 (57.14)	0.631
Anti-β2-GP I IgG	0.141±1.43 (0.42)	1.58±6.69 (-1.04)	31.53±35.08 (28.53)	0.077

V: Valine, L: Leucine. Kruskal Wallis test.

or VV, VL, LL genotypes were similar ($p=0.940$, $p=0.882$, and $p=0.355$, $p=0.861$, respectively). Interestingly, V allele and VV genotype frequencies were significantly higher in patients with thrombocytopenia ($p=0.037$ and $p=0.014$, respectively) (Table 4). This finding was significant in patients with primary APS ($p=0.04$) (Table 5).

Discussion

The frequency of β2-GP I Val247Leu polymorphism in patients with APS was shown to be different among various ethnic populations. In this study, we analyzed the

Table 4. Clinical manifestations and allele, genotype frequencies.

	Thrombosis (n=83)		Pregnancy morbidity (n=61)		Thrombocytopenia(n=83)	
	Yes (n=76)	No (n=7)	Yes (n=26)	No (n=35)	Yes (n=15)	No (n=68)
VV	11/76 (14.5%)	2/7 (28.6%)	4/26 (15.4%)	4/35 (11.4%)	6/15 (40.0%)	7/68 (10.3%)
VL	30/76 (39.5%)	1/7 (14.3%)	9/26 (34.6%)	14/35 (40.0%)	3/15 (20.0%)	27/68 (39.7%)
LL	35/76 (46.1%)	4/7 (57.1%)	13/26 (50.0%)	7/35 (48.6%)	6/15 (40.0%)	34/68 (50.0%)
p	0,355				0,014*	
	Yes (n=152)	No (n=14)	Yes (n=52)	No (n=70)	Yes (n=30)	No (n=136)
V	52/152 (34.2%)	5/14 (35.7%)	17/52 (32.7%)	22/70 (31.4%)	15/30 (50.0%)	41/136 (30.1%)
L	100/152 (65.8%)	9/14 (64.3%)	35/52 (67.3%)	48/70 (68.6%)	15/30 (50.0%)	95/136 (69.9%)
p	0.940		0.882		0.037*	

V: Valine, L: Leucine. Chi-square test.

B2-GP I Val247Leu polymorphism and clinical manifestations of APS

In patients with a history of thrombosis or pregnancy morbidity, V and L allele frequency

expression of Val247Leu polymorphism in APS patients and healthy individuals, its relationship with anti-β2-GP I antibodies and clinical manifestations.

Table 5. Thrombocytopenia in primary APS and APS with SLE.

				Thrombocytopenia		Total	p
				No	Yes		
Primary APS		VV	N (%)	7 (13)	6 (42,9)	13 (19,1)	0,040*
		VL	N (%)	19 (35,2)	3 (21,4)	22 (32,4)	
		LL	N (%)	28 (51,9)	5 (35,7)	33 (48,5)	
	Total		N	54	14	68	
APS with SLE		VL	N (%)	7 (58,3)	1 (33,3)	8 (53,3)	0,569**
		LL	N (%)	5 (41,7)	2 (66,7)	7 (46,7)	
	Total		N	12	3	15	

V: Valine, L: Leucine, APS: Antiphospholipid syndrome, SLE: Systemic lupus erythematosus. *Chi-square test, **Fisher's exact test.

In our study, the allele and genotype frequencies were similar in patients with APS and healthy individuals as reported in previous studies in Caucasian populations. Hirose et al studied Caucasian, African American and Asian ethnic groups together. They reported that the V allele was expressed most often among Caucasians, less among African Americans and least among Asian healthy individuals (68% vs 46% vs 24%). When they compared allele and genotype frequencies in APS patients, only in Asian patients with APS the expression of V, especially VV genotype, was significantly associated with the presence of anti- β_2 -GP I antibodies. No significant differences in allele or genotype frequencies were among the Caucasian or the African American patients [16]. Camilleri et al, did not find any difference in allele or genotype frequencies between patients or control groups in British patients with thrombosis or any relation to anti- β_2 -GP I antibodies. They also reported high frequency of Val allele in the healthy Caucasian population [17]. Swadzba et al, showed that among the Caucasian Polish population of autoimmune patients Val247Leu polymorphism had the same distribution as in

healthy subjects and did not influence the production of anti- β_2 GPI antibodies [18]. Pardos-Gea et al, concluded no association between Val247Leu polymorphism and primary APS among Spanish Caucasians and showed no correlation of Val247Leu genotypes with anti- β_2 GPI antibodies [19].

We did not find any association between Val247Leu gene polymorphism and anti- β_2 -GP I antibodies. Even though this result is in line with previous studies in Caucasian populations [16-19] the limited number of patients with anti- β_2 -GP I antibodies preclude us from drawing definite conclusions.

Regarding clinical findings, we did not find any association between thrombosis or pregnancy morbidity and Val247Leu polymorphism. Previous studies in Caucasian populations reported similar findings [17-19]. Interestingly, V allele and VV genotype frequencies were found to be significantly higher in primary APS patients with thrombocytopenia; which was not analyzed in previous studies. This could offer further insight into the pathogenesis of thrombocytopenia in APS.

Studies in Asian and South American populations have suggested clinical and

serological associations between APS and β 2-GP I gene Val247Leu polymorphism. Yasuda et al showed that the Val247Leu polymorphism was correlated with anti- β 2-GP I antibody production in patients with primary APS and V allele could be important in the formation of β 2-GP I antigenicity in Japanese patients [12]. Even though Prieto et al found no significant differences in the genotype expression between primary APS patients and control group, they suggested that the VV genotype could play a role in the generation of anti- β 2-GP I antibodies and in the expression of arterial thrombosis in primary APS in Mexican patients [13]. Palomo et al showed that, the Val/Leu 247 and Tryptophan/Serine 316 polymorphisms were not related to the presence of anti- β 2-GP I antibodies in unselected Chilean patients with venous and arterial thrombosis, but they were significantly associated with venous and arterial thrombosis [14]. Pernambuco-Climaco et al, showed significantly high frequency of V allele and VV genotype in Brazilian patients with APS [15].

Clarify controversial data from different ethnic backgrounds, two meta-analysis have been published. The first meta-analyses including aforementioned studies [11-13, 16, 18-20] by Chamorro et al, concluded VV genotype was associated with a significant excess risk to suffer from APS and among patients with APS, to have anti- β 2-GP I antibodies but no definite conclusion could be made regarding association of this polymorphism with thrombosis among APS patients. Regarding ethnicity analysis, no association was found between the possession of the VV genotype in the Caucasians and the presence of APS. It was emphasized that low number of studies, different ethnicity and mixing of patient with primary APS and APS associated to autoimmune diseases precluded drawing conclusions regarding specific

ethnicity [21]. The second meta-analysis by Lee et al, included same studies [11, 12, 16-18, 20] showed Val247Leu polymorphism was associated with susceptibility to APS and thrombosis and anti- β 2-GP I positivity. Frequencies of Val247Leu polymorphism were different among races; the results from the study with the biggest population were in Caucasians and negative [18] so limited data prevented ethnic-specific meta-analyses [22]. These have shown the need for studies in different ethnic groups to clarify the role for β 2-GP I polymorphism [22]. This is the first study analyzing Turkish population. In summary, we aimed to investigate the frequency of Val247Leu polymorphism in Turkish APS patients and healthy individuals; and its association with clinical manifestations and anti- β 2-GP I antibodies. Similar to previous studies in Caucasians, allele and genotype frequencies were similar between APS patients and healthy individuals. There was no association between different allele or genotypes and anti- β 2-GP I antibodies, thrombosis, pregnancy morbidity. We found significant association between V allele and VV genotype and thrombocytopenia in primary APS patients.

To our knowledge this is the first time this has been reported. Limited number of patients and healthy controls is one of the drawbacks of this study. Further studies are needed to investigate the β 2-GP I antigen polymorphism and its role in pathogenesis of APS and thrombocytopenia. Unfortunately, we were not able to measure lupus anticoagulant, anti-cardiolipin IgM, and IgG levels simultaneously at the time of this study. Since these antibodies may fluctuate over time, we did not choose to make an assumption regarding this antigen polymorphism and previous antibody results

for lupus anticoagulant and anti-cardiolipin antibodies based on chart review.

Further studies are needed to investigate the β 2-GP I antigen polymorphism and its relationship with anti- β 2-GP I and anti-cardiolipin antibodies and lupus anticoagulant levels and its role in the pathogenesis of APS and thrombocytopenia.

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Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical Statement: The study was approved by Istanbul University Ethics Committee for Clinical Studies (Date and number: 2008/2157)

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