

The association of beta-fibrinogen 455 G/A gene polymorphism with left atrial thrombus and severe spontaneous echo contrast in atrial fibrillation

Atriyal fibrilasyonda beta-fibrinojen 455 G/A gen polimorfizmi ile sol atriyal trombüs ve ciddi spontan eko-kontrast arasındaki ilişki

Volkan Bozdemir, Önder Kıymılı, Bahri Akdeniz, Ayfer Ügüenalp, Abdurrahman Aslan, Veli Kala, Erdem Özel, Ömer Şenarşlan, Özer Badak, Nezih Barış, Sema Güneri*

From Departments of Cardiology and * Medical Genetics, Faculty of Medicine, Dokuz Eylül University, İzmir, Turkey

ABSTRACT

Objective: The role of coagulation parameters left atrial thrombus formation in atrial fibrillation has not been investigated before. We aimed to investigate the association between the beta-fibrinogen gene polymorphism or glycoprotein IIIa gene polymorphism and presence of left atrial (LA) thrombus or spontaneous echo contrast (SEC) in patients with atrial fibrillation (AF).

Methods: Forty-seven patients with AF, in whom transesophageal echocardiography was performed, were included to this cross-sectional observational study. Patients were divided in two groups; those with LA thrombus (n=24) were assigned to group 1 and those without thrombus in group 2 (n=23). DNA analysis was conducted to determine gene polymorphism in all patients. Mann-Whitney U test or Chi-square tests were used for statistical analysis

Results: There were no significant differences between groups regarding to demographic and clinical characteristics. The frequency of beta-fibrinogen 455 G/A polymorphism was higher (37.5%) in group 1 as compared to group 2 (15.1%) but it did not reach statistical difference (p=0.23). When we added patients with severe SEC in the study group (patients with severe SEC and/or thrombus n=27) the difference (44.40%-10%) reached the statistical difference (p=0.01). Glycoprotein IIIa PI A1/A2 polymorphism was not different between groups with (p=0.82) or without SEC (p=0.73).

Conclusion: In patients with atrial fibrillation, beta-fibrinogen 455 G/A gene polymorphism is associated with the presence of left atrial thrombus and severe SEC. Beta-fibrinogen 455 G/A gene polymorphism may be a promising marker for the prediction of thromboembolism risk in patients with atrial fibrillation. (*Anadolu Kardiyol Derg 2010; 10: 209-15*)

Key words: Atrial fibrillation, thrombus, left atrium, gene polymorphism, beta-fibrinogen, glycoprotein IIIa

ÖZET

Amaç: Atriyal fibrilasyonda sol atriyal trombüs oluşması açısından koagülasyon parametrelerinin rolü yeterince araştırılmamıştır. Atriyal fibrilasyonlu hastalarda sol atriyal trombüs ile veya onun öncüsü olduğu düşünülen spontan eko-kontrast varlığı (SEK) ile beta fibrinojen gen polimorfizmi ile trombosit glikoprotein IIIa gen polimorfizmi arasındaki ilişkiyi araştırmayı amaçladık.

Yöntemler: Transözofageal ekokardiyografi yapılan atriyal fibrilasyonlu 47 hasta enine kesitsel gözlemsel çalışmamıza dahil edildi. Hastalar 2 gruba ayrıldı. Sol atriyal trombüsü olanlar Grup 1'i (n=24) ve trombüsü olmayanlar grup 2'yi (n=23) oluşturdu. Beta fibrinojen gene polimorfizm veya glikoprotein IIIa gen polimorfizmlerini saptamak için genetik analizler yapıldı. İstatistiksel analizde Mann Whitney U ve Ki-kare testleri kullanıldı.

Bulgular: Demografik ve klinik özellikler bakımından gruplar arasında fark saptanmadı. Beta fibrinojen 455 G/A gen polimorfizm sıklığı grup 1'de (%37.5) grup 2'ye göre (%15) istatistiksel anlamlılığa ulaşmayacak şekilde (p=0.23) daha yüksek saptandı. Çalışma grubuna ciddi SEK'i olan hastalar da eklendiğinde (trombüs ve ciddi SEK n=27) 2 grup arasındaki fark (%44.4-%10) istatistiksel anlamlılığa ulaşmaktaydı (p=0.01). Glikoprotein IIIa PI A1/A2 polimorfizmi ise gruplar arasında (p=0.73) SEK eklense de (p=0.82) farklı bulunmadı. Grup 1'de grup 2'ye göre mitral yetmezliği skoru daha düşük olma eğiliminde (p=0.08), SEK skoru ise anlamlı olarak daha yüksek idi (p=0.03).

Address for Correspondence/Yazışma Adresi: Dr. Bahri Akdeniz, Dokuz Eylül Üniversitesi, Tıp Fakültesi, Kardiyoloji Anabilim Dalı Inciraltı 35340 İzmir, Turkey
Phone: +90 232 412 41 08 Fax: +90 232 279 25 65 E-mail: bahri.akdeniz@deu.edu.tr

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Sonuç: Atriyal fibrilasyonlu hastalarda beta fibrinojen 455 G/A gen polimorfizmi varlığı sol atriyal trombüs ve ciddi spontan eko-kontrast varlığı ile ilişkilidir. Glikoprotein IIIA PI A1/A2 polimorfizminin ise sol atriyal trombüs ile ilişkisi yoktur. Atriyal fibrilasyonlu hastalarda tromboemboli riskinin belirlenmesinde beta fibrinojen gen 455 G/A polimorfizmi umut vaat eden, bir gösterge olabilir. (*Anadolu Kardiyol Derg 2010; 10: 209-14*)
Anahtar kelimeler: Atriyal fibrilasyon, trombüs, sol atriyum, gen polimorfizmi, beta-fibrinojen, glikoprotein IIIa

Introduction

Atrial fibrillation (AF) is the most commonly encountered rhythm problem in adult population (1). It is associated with increased long-term risk of stroke as well as heart failure and death (2, 3). Compared to healthy population, nonvalvular AF has a 2-7 fold increased risk of ischemic stroke (3-5). Previous stroke, advanced age, hypertension, diabetes mellitus and heart failure are traditional risk factors for stroke in patients with AF.

Left atrial appendix (LAA) is the most suitable region for the development of stasis and thrombus formation in the heart. Transesophageal echocardiography (TEE) is required for imaging left atrial appendage, which can not be easily seen in transthoracic echocardiography (6). The echo-dens material in the left atrium, named spontaneous echo-contrast (SEC), may be seen in some of the patients with AF during TEE procedure (7). Fibrinogen mediated aggregation of erythrocytes is thought to be responsible for SEC formation (8).

The mechanisms of thrombus formation in AF are still investigated. Virchow triad including stasis, endothelial dysfunction and hypercoagulopathy acts as an important factor for thrombus formation in left atrium. The stasis developed due to loss of atrial contractility is the most important reason (9). However, the role of hypercoagulopathy for left atrial thrombus formation has not been investigated adequately until now. A few biochemical markers influencing the coagulation cascade were investigated in terms of the role of those on thrombus formation or embolic events in limited number of studies. However, these biochemical markers may be affected by many individual factors. This handicap limits the diagnostic or prognostic use of these markers. On the other hand, a genetic analysis which is not affected by other individual alterations and medications has more consistent and favorable outcomes compared to simple biochemical analysis of surrogate markers. It is also possible to make a retrospective analysis in patients whose thrombus had been detected before.

Fibrinogen plays an active role during the coagulation process. Increased plasma fibrinogen levels were shown to be associated with the coronary heart disease (10), peripheral artery disease and venous thrombosis (11-13). Beta-fibrinogen 455 G/A polymorphism is a gene mutation that may lead to alterations in the activity of fibrinogen. Previous study revealed the increased fibrinogen activity in the presence of homozygote A/A allele (14-16). It was determined that the increased A/A allele is associated with increased cardiovascular events (17, 18) and increased prevalence of lacunar infarct in brain (19). However, conflicting results were also reported (20)

Glycoprotein PLA1/A2 polymorphism is another gene mutation, which is responsible for predisposition to thrombosis through change in the activity of a glycoprotein IIIa receptor located on the surface of a thrombocyte. Although thrombocyte functions are normal in the presence of PLA1 allele, increased thrombotic events, especially increased acute coronary syndrome risk was reported in young patients with PLA2 allele, (21, 22). Nonetheless, the opposite result was also reported (23).

The association of these 2 mutations with left atrial thrombus formation has not been investigated before. We aimed to evaluate the association between polymorphisms at beta fibrinogen 455 G/A gene and glycoprotein PLA / A2 gene and the presence of left atrial thrombus the in AF.

Methods

Patients

All patients with AF who underwent TEE in our cardiology unit between April 2006 and August 2007 in order to detect left atrial thrombus before planned cardioversion were assessed. Cross-sectional and observational study was designed. Patients with left atrial thrombus were selected as the study group. Patients without thrombus on TEE during the same assessment period were selected as control group.

The blood samples were collected from all patients after TEE procedure. Additionally, we screened the patients, who underwent TEE for last 4 years in our echocardiography laboratory. In this retrospective analysis, all patients with thrombus found appropriate with the inclusion criteria of study protocol were also assessed and those who could have been reached (address or phone number) were recruited the hospital. Appropriate patients were also assigned to study group and their blood samples were drawn. All study participants were informed about the procedures. The study protocol was approved by the local Ethics committee.

Exclusion Criteria: All patients with paroxysmal AF were excluded. Other exclusion criteria are as follows: Rheumatic valvular heart disease, severe congenital heart disease, patients receiving anticoagulant drugs, patient with a larger left atrium (>5.5 cm) and those with a low ejection fraction (EF <30%).

Echocardiography

Conventional transthoracic echocardiography (TTE) and TEE were performed using Hewlett Packard, Agilent Technologies Sonos 4500 (Palo Alto, California, USA) system in all patients before cardioversion. The transthoracic echocardiographic

measurements were obtained from parasternal long-axis view by 2-D targeted M-mode tracings according to the recommendations of the American Society of Echocardiography. Left atrial (LA) diameter, left ventricular end-systolic and end-diastolic diameter, left ventricular ejection fraction (LVEF) (according to Teichholz formula and modified Simpson's method in patients with segmental wall motion abnormalities) were measured. The degree of mitral regurgitation (MR) was determined according to American College of Cardiology / American Heart Association criteria (24).

Transesophageal echocardiography was performed with multiplane probes with a 7.0 MHz. transducers. All TEE procedures were performed by experienced cardiologists (VB, BA, NB, OB) for the presence of intracardiac thrombi. In order to view the maximal size and to obtain the highest resolution of the left atrial appendage, the most appropriate section was used for the analysis. Special attention was paid to assess the presence or absence of left atrial thrombi and degree of spontaneous echo-contrast (SEC) during TEE examination. A thrombus was considered to be present when a well-circumscribed echodense intracavitary mass that was acoustically distinct from the underlying endocardium was detected. The degree of spontaneous echo-contrast was graded according the following criteria: mild was defined as minimal echogenicity located in the left atrial appendage or sparsely distributed in the main cavity of the left atrium, which was possible to detect only transiently during the cardiac cycle, but imperceptible at operating gain settings for two-dimensional echocardiographic analysis. 'Moderate' SEC was defined as a dense, swirling pattern in the left atrial appendage, generally associated with somewhat lesser intensity in the main cavity, which may fluctuate in intensity but detectable constantly through the cardiac cycle. 'Severe' SEC was defined as an intense, echo density and a very slow swirling pattern in the left atrial appendage, usually with similar density in the main cavity (25).

Genetic analysis

Blood samples were drawn from all patients via peripheral venous route and all samples stored in deep-freeze. For DNA extraction, a 100 µl of dissolved blood sample were centrifuged at 3000 rpm with a solvent solution for 5 minutes. After receiving the supernatant, recentrifugation process was performed with a substitution of a 1 ml solvent solution at 12000 rpm, and supernatant of this solution were drawn. We used PCR and reverse hybridization to genotype samples for analyse of beta fibrinogen gene polymorphism and glycoprotein P1 A1/A2 polymorphism. Assay includes three steps: Genomic DNA was isolated from peripheral blood leukocytes with use of a commercially available reagent (Invisorb Spin Blood Mini Kit: Invitex, Biodesign GmbH, Berlin, Germany). DNA samples were amplified biotinylated in multiplex PCR reactions for 35 cycles of denaturation at 94°C for 15 s, annealing at 58°C for 30 s, and extension at 72°C for 30 s, followed by a final extension step at 72°C for 3 min., and hybridized for 30 min at 45°C to a membrane test strip. Finally, the amplification products are selectively hybridized to a test strip, which contains allele-specific (wild type and mutant) oligonucleotide probes immobilized as an array of parallel lines. Bound biotinylated sequences are detected using streptavidin-alkaline phosphatase and colour substrates (CVD Strip Assay; ViennaLab, Vienna, Austria).

Statistical analysis

SPSS 11.0 for Windows (Chicago, IL, USA) was used for the statistical analysis. Continuous variables are presented as a mean or median regarding to parametric or nonparametric condition. Differences between groups were assessed by Mann-Whitney U test for continuous variables. Comparison of categorical variables was generated by Chi-square or Fisher Exact tests analysis. All tests were two sided. A p value <0.05 was considered significant.

Table 1. Demographic and clinic characteristics of groups

Variables	All study population n=47	Pts with thrombus n=24	Pts without thrombus n=23	p*
Age, years	68 (54-82)	71 (58-82)	67 (54-82)	0.25
Sex, female/male	26/21	15/9	11/12	0.31
DM, %	27	29.1	26	0.81
HT, %	57.4	54.1	60.8	0.64
History of CHD, %	23	20.8	26	0.67
History of ischemic stroke / TIA, %	12	16.6	8.6	0.41
Diameter of LA, cm	4.5 (3.6-5.2)	4.5 (3.9-5.2)	4.5 (3.6-5.2)	0.53
LVEF, %	60 (30-79)	57.5 (30-73)	60 (45-79)	0.44

Data are presented as proportions/percentages and median (min-max) values

*Chi-square and Mann Whitney U t tests

CHD - coronary heart disease, DM - diabetes mellitus, HT - hypertension LA - left atrium, LVEF - left ventricular ejection fraction, pts - patients, TIA - transient ischemic attack

Results

A total 47 patients (median age: 68 years) were included to the study. A few of the patients (12%) had a history of stroke or TIA. Study group (patients with thrombus) consisted of 24 patients (median age: 71 years) and control group consisted of 23 patients (mean age: 67 years). The majority (96%) of the patients had SEC on TEE; 72.3% had mild SEC, 8.5% - moderate and 14.8% - severe. The demographic and clinical characteristics of all patients are summarized in Table 1.

There were no significant differences between groups regarding to age, sex, and the prevalence of hypertension, diabetes mellitus as well as LVEF and LA diameter (Table 1). If we analyzed both groups in terms of the grade of SEC and MR, patients with LA thrombus had also significantly higher prevalence of SEC compared to patients without thrombus (p=0.03) (Table 2). The mean degree of MR was lower in patients with thrombus group but the difference did not reach a statistical significance (p=0.08) (Table 2).

The associations between beta fibrinogen 455 G/A polymorphism and the presence of thrombus or SEC

Beta- fibrinogen 455G/A polymorphism was detected at 14 patients (29%) in whole study population, 12 of them were heterozygote (G/A) and 2 out of them were homozygote (A/A) type. LA thrombus was present in both patients with homozygote (A/A) type. Beta-fibrinogen 455G/A polymorphism was present at 9 patients (37.5%) in patients with thrombus and 5 patients at patients without thrombus (15.1%). Although the prevalence of this polymorphism was higher in study group, the difference did not reach the statistical significance (p=0.23) (Table 1).

We also analyzed the patients with severe SEC, considered to have predisposition to thrombus formation. We assessed these patients (n=7) in a new group by adding to patients with LA thrombus. Beta-fibrinogen 455G/A polymorphism was present in 12 out of 27 patients with severe SEC and/or thrombus (44%), and only 2 patients without severe SEC and thrombus (10%) (Table 3). Thus, if we added the patients with severe SEC on the study group (patients with severe SEC and/or thrombus) the difference reached the statistical difference (OR=7.27, 95% CI: 1.20-55.42) (p=0.01) (Table 3).

The frequency of patients with previous stroke or TIA was similar in patients with (7.1%) and without (15.1%) beta-fibrinogen 455G/A polymorphism (p=0.46).

The associations between glycoprotein IIIa PIA2 polymorphism and the presence of thrombus and/or severe SEC

Glycoprotein IIIa PIA2 polymorphism was present 11 patients (23.4%) in whole study population. Ten out of them were heterozygote, and 1 of them was homozygote polymorphism which was in the control group. The prevalence of glycoprotein IIIa PIA2

Table 2. The comparison of study and control group regarding to SEC, the degree of MR, beta-fibrinogen 455 G/A polymorphism, glycoprotein IIIa PIA2 polymorphism

Variables	Pts with thrombus (n=24)	Pts without thrombus (n=23)	*p
SEC	1 (1-3) 1.58±0.82	1 (0-3) 1.17±0.69	0.03
MR	2 (1-4) 1.79±0.72	2 (0-3) 2.04±0.70	0.08
Beta fibrinogen G/A polymorphism, n (%)	9 (37.5)	5 (15.1)	0.23
Glycoprotein IIIa PIA2 polymorphism, n (%)	5 (20.8)	6 (26)	0.73

Data are presented as mean±SD, proportions/ percentages and median (min-max) values
*Chi-square and Mann Whitney U t tests
MR - mitral regurgitation, pts - patients, SEC - spontaneous echo-contrast

Table 3. The associations between beta-fibrinogen 455G/A polymorphism, glycoprotein IIIa PIA2 polymorphism and the presence of thrombus and/or SEC

Variables	Pts with thrombus or severe SEC n=27	Pts without thrombus or severe SEC n=20	*p
Beta-fibrinogen G/A polymorphism, n (%)	12 (44.4)	2 (10)	0.01
Glycoprotein IIIa PIA2 polymorphism, n (%)	6 (22.2)	5 (25)	0.82

Data are represented as proportions/ percentages
*Chi-square test
pts - patients, SEC - spontaneous echo-contrast

polymorphism was 20.8% (n=5) in study group, and 26% (n=6) in control group. There was no difference between patients with and those without LA thrombus regarding to prevalence of glycoprotein IIIa PIA2 polymorphism (p=0.73) (Table 2). When we divided the groups with and without LA thrombus and/or severe SEC, the difference remain nonsignificant between groups in terms of glycoprotein IIIa PIA2 polymorphism (p=0.82) (Table 3). The frequency of patients with previous stroke / TIA was also similar between patients with (18.1%) and without (11.1%) glycoprotein IIIa PIA2 polymorphism (p=0.61).

Discussion

In this study, beta-fibrinogen 455G/A polymorphism is found to be associated with the presence of thrombus and/or severe SEC formation in left atrium. The presence of this polymorphism has a 7.2 fold increased risk of LA thrombus or severe SEC in patients with AF. It is also related with LA thrombus alone but the difference did not reach the significance. A low sample size in the study may be responsible for nonsignificance. When we added the patients with severe SEC to the study group, the difference reached the statistical difference.

Beta-fibrinogen 455G/A polymorphism was detected in 29% of whole study population. LA thrombus was present in patients with homozygote (A/A) type. The number of patient who has homozygote A/A allele was not frequent. Therefore, we could not exactly determine the LA thrombus risk among homozygote individuals separately.

Some problems involving the coagulation cascade are also suggested to be related with the LA thrombus formation and systemic embolism. Several biochemical markers involving the coagulation cascade were investigated in a few clinical studies. (26, 27). However, these markers may change instantaneously, and they may also be affected by many other conditions. Recent studies revealed the relationship between beta-fibrinogen 455G/A polymorphism and stroke or myocardial infarction (17-19). Beta-fibrinogen 455G/A polymorphism was also shown to be associated with the increased erythrocyte aggregation and predisposing the development of stasis (28). The presence of defective fibrinogen may lead to change the blood viscosity, thereby, may precipitate a thrombus formation in surfaces, which may prone to stasis. So, it is reasonable that beta-fibrinogen 455G/A polymorphism may predispose to LA thrombus formation by leading the development of stasis.

SEC is thought to be consequence of aggregation of erythrocyte with blood proteins especially fibrinogen. The severity SEC was shown to be increased with the elevated fibrinogen levels (8). Severe SEC, a marker of atrial stasis, is suggested to associate with thrombus formation and increased systemic embolism risk (19, 25). Fibrinogen may be the key element for the development of thrombus and SEC formation. The presence of defective fibrinogen may cause atrial stasis and thrombocyte activation or both, therefore may play a role for the development of LA thrombus and SEC.

It is reasonable to explain the increased left atrial thrombus risk of homozygote persons carrying A allele due to the increased fibrinogen levels. However, the reason of increased thrombus risk in patients who were heterozygote for the fibrinogen 455G/A allele is unclear. Some plausible mechanisms including abnormal response to stress and transient exaggerated production rather than a permanent increase of fibrinogen blood levels are claimed for the increased thrombus risk among patients with heterozygote polymorphism. Increased fibrinogen levels in response to exercise are shown among patients with beta-fibrinogen 455G/A polymorphism (29). The presence of A allele may lead to increase in high molecular weight type of fibrinogen, which may tend to have an increased activity of it (14).

We prefer a relatively homogenous group in order to assess the role of coagulation elements on thrombus formation. Other confounding factors predisposing the thrombus formation including severe mitral stenosis, left ventricular systolic dysfunction and enlarged left atrium were excluded from this study.

The relationship between glycoprotein IIIa PIA2 polymorphism and thrombosis risk is controversial. Some of the previous studies suggested the increased risk among patients with glycoprotein IIIa PIA2 polymorphism (21, 22). However, one study did not confirm this finding (23). The absence of relationship between this polymorphism and thromboembolism risk may be due to the fact that glycoprotein IIIa is present on the surface of activated thrombocyte, whereas it is not present in normal circulation. It may suggested that it contributes become a complex situation of dysfunction rather than play an active initiative role in the pathogenesis of thrombus formation. Glycoprotein IIIa PIA2 polymorphism was shown to be associated with acute thrombotic events rather than the initiation of atherosclerosis at previous post-mortem studies. These findings suggest that this polymorphism is related to increased thrombocyte in response to vessel injury. Our findings showed that left atrial thrombus formation in AF may be due to loss of preventive properties of atrial endothelium against coagulation rather than the presence of apparent injury in endothelium. Concordantly, the relative inefficacy of anti-platelet regimen compared to anticoagulant medication in terms of the treatment of left atrial thrombus is thought that coagulation system is more important in the pathogenesis of this process. In our study, a genetic defect leading to abnormalities of coagulation system is found to be associated with thrombus/or SEC formation, whereas other genetic defect leading to the abnormalities of thrombocyte activation did not.

The negative correlation between the MR and presence of thrombus has been reported previously (30). This study confirmed this finding. Although it did not reach statistical significance, MR score was lower in patients with left atrial thrombus. This study confirmed the higher prevalence of SEC in patients with LA thrombus (17, 31).

Study limitations

The sample size may be relatively low, and may affect the outcome of this study. It was difficult to find patients with thrombus and archive screening was required to increase the number of patient with thrombus. Although this situation does not change the genetic study, the retrospective identification of some cases with SEC or thrombus may limit this study.

Our study is an observational study, thus, the experimental or prospective clinical studies with larger sample size are required in order to confirm our observations.

The whole study population consisted of patients with persistent AF, who underwent TEE before a cardioversion procedure. Thus, the outcomes of this study cannot be generalized to the permanent AF population. However, most patients in this study similarly to those with permanent AF had a long AF duration.

The absence of the assessment of serum fibrinogen levels concordant with beta-fibrinogen 455G/A polymorphism may limit

the outcomes. However, we know that blood fibrinogen levels increased only in the presence of homozygote A/A allele (14-16). Additionally blood fibrinogen is also an acute phase reactant, therefore, instantaneous changes may be seen. It is also affected many other conditions. Finally, it is not suitable for the assessment of patients in whom TEE was performed previously.

Conclusion

Beta-fibrinogen 455G/A polymorphism, even in presence of heterozygote allele, is associated with the presence of thrombus and/or SEC in left atrium in patients with AF, whereas, this association was not detected with glycoprotein IIIa PIA2 polymorphism. Beta-fibrinogen 455G/A polymorphism may be a promising marker for risk of left atrial thrombus. It may help determine thromboembolic risk status of patients with AF and may help to decide to requirement of intensive anticoagulant therapy.

Conflict of interest: None declared

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