Factor XIII Val34Leu polymorphism modulates the prothrombotic and inflammatory state associated with atrial fibrillation

Francisco Marín a,*, Javier Corral b, Vanessa Roldán c, Rocío González-Conejero b, María Luz del Rey b, Francisco Sogorb a, Gregory Y.H. Lip d, Vicente Vicente a

a Cardiology Service, Hospital General Universitario, C/Pintor Baeza s/n, 03010 Alicante, Spain
b Hematology Service, Hospital Morales Meseguer, Murcia, Spain
c Hematology Unit, Hospital de San Vicente, Alicante, Spain
d Haemostasis, Thrombosis and Vascular Biology Unit, University Department of Medicine, City Hospital, Birmingham, UK

Received 18 May 2004; received in revised form 19 May 2004; accepted 1 June 2004
Available online 31 July 2004

Abstract

Atrial fibrillation (AF) has been shown to confer a prothrombotic or hypercoagulable state, which could be related to inflammation. Factor XIII (FXIII) catalyses the cross-linking of fibrin monomers, increasing clot resistance; specifically, a common polymorphism, Val34Leu, in the FXIII-A subunit gene has been associated with more rapid FXIII activation. We hypothesised a role for this polymorphism in the prothrombotic state and inflammation in AF, and tested this hypothesis by measurement of indices of coagulation (tissue factor (TF) and fibrinogen), inflammation (interleukin-6 (IL6)) and platelet activation (soluble P selectin (sPsel)).

Methods. – We studied 90 stable outpatients (73 ± 8 years) with persistent AF. The FXIII Val34Leu polymorphism was determined by polymerase chain reaction–allelic specific restriction assay (PCR–ASRA). Prevalence of Val34Leu polymorphism of patients was compared to 585 unrelated subjects from the same geographical area. Plasma fibrinogen (Clauss), TF, IL6 and sPsel (all ELISA) were quantified in patient group. Research indices were compared to 74 controls in sinus rhythm with similar clinical characteristics.

Results. – There were no statistical differences in FXIII polymorphism prevalence between AF patients and controls. Patients carrying the Leu34 allele had higher plasma levels of TF, IL6 and sPsel (all P < 0.05) compared to controls. Plasma IL6 and TF levels were significantly correlated (Spearman coefficient, r = 0.33, P < 0.01). On multivariate analysis, the Leu34 allele was independently associated with IL6 levels (P < 0.01), whereas TF levels were only associated with IL6 concentrations. However, sPsel and fibrinogen levels were not related to Leu34 allele.

Conclusion. – FXIII Val34Leu polymorphism was independently associated with IL6 levels in AF. The Leu34 allele may potentially influence the prothrombotic state in these patients by modulating the inflammatory state.

© 2004 Elsevier Ltd. All rights reserved.

Keywords: Atrial fibrillation; Inflammation; Tissue factor; Polymorphism

1. Introduction

Atrial fibrillation (AF) is a common cardiac arrhythmia, which confers a prothrombotic or hypercoagulable state, that may increase the risk of stroke and thromboembolism [1,2]. This prothrombotic state has been associated with the presence of left atrial thrombus and spontaneous echo contrast [3]. Nonetheless, the precise mechanistic pathway(s) leading to the prothrombotic state in AF remain to be elucidated, although inflammatory stimuli have been hypothesised as a possible mechanism.

Interleukin-6 (IL6) is a circulating cytokine produced by monocytes, macrophages, T lymphocytes and endothelial cells, which may induce a prothrombotic state by increasing expression of fibrinogen, tissue factor (TF), factor VIII (FXIII) and von Willebrand factor, as well as by activating endothelial cells and increasing platelet production [4]. Indeed, high levels of IL6 have been found in AF, suggesting the presence of an inflammatory state, which appears to be more related to clinical variables of the patients rather than to the presence of AF per se [5]. However, controversial data
exist about the relationship between AF and inflammatory state, as C-reactive protein (CRP), an established marker of inflammation, has been independently associated not only with the presence of the arrhythmia but may predict its development [6,7]. Additionally, AF has been associated with abnormal platelet activation [8] and high TF [9]. Certainly, TF is a transmembrane procoagulant glycoprotein and a member of the cytokine receptor family, which is expressed by a wide type of cells, and responsible for the initiation of haemostasis in vivo [10]. The expression of TF by monocytes is associated with an activation of the immune response, and indeed, CRP can induce TF expression by human monocytes [11].

Coagulation FXIII is a tetrameric structure consisting of 2A (active) and 2B subunits. Calcium and thrombin activate FXIII in the final phase of the coagulation process. FXIII plays an essential role in the coagulation process, as activated FXIII catalyses the formation of γ-glutamyl-lysine bonds between fibrin and α₉-plasmin inhibitor, increasing the resistance of fibrin to degradation [12]. A common G to T polymorphism in exon 2 of the FXIII-A subunit gene causes a valine to leucine change at position 34, three amino acids upstream to the thrombin cleavage site [13]. However, there are controversial data about the role of this polymorphism in cardiovascular disease [14–16], although it is universally accepted that the Val34Leu polymorphism is one of the most important functional polymorphisms described so far. Indeed, the Leu34 variant displays an increased rate of FXIII activation by thrombin [17], which results in an increased FXIII-specific transglutaminase activity [18], with an increased and faster rate of fibrin stabilisation [19,20]. Thus, fibrin clots formed in the presence of Leu34 are more resistant to fibrinolysis—as assessed by thromboelastography [21] and display more resistance to fibrinolytic therapy in patients with acute myocardial infarction [22]. In accordance, thrombi formed in subjects carrying the FXIII Leu34 variant would take a much longer time for removal from the vascular system. Finally, recent reports support an association between transglutaminase activity and inflammation [23].

We hypothesised a role for the Val34Leu polymorphism in the prothrombotic and inflammatory states (which appear to be linked [24]) and in AF, by measurement of indices of coagulation (TF and fibrinogen), inflammation (IL6) and platelet activation (soluble P-selectin, sPsel) in 90 patients with persistent AF.

2. Patients and methods

2.1. Patients and controls

We have studied 90 consecutive Caucasian patients (44 male; mean age ± S.D.: 72.5 ± 8.4 years) with non-rheumatic persistent AF lasting more than 4 weeks (as documented by electrocardiography), who were referred by general practitioners or cardiologists to our anticoagulation clinic for oral anticoagulation. These patients were all those recruited in one of the centres (Hospital de San Vicente, Alicante, Spain) included in a previous cohort of non-anticoagulated AF patients [5]. None of the patients had previously been taking anticoagulant therapy, although 46 were taking aspirin. Risk factors for thromboembolic events were recorded—both clinical (age, sex, hypertension, diabetes, heart failure and previous embolism) and echocardiographical variables (left atria diameter, spontaneous echodensity and left ventricular ejection fraction). We excluded patients with recent (<3 months) venous or systemic thromboembolism, myocardial infarction, stroke or acute coronary syndrome, infection or inflammatory disease and/or surgery; malignancy and renal/liver impairment. Finally, we also excluded patients with valvular heart disease and those being treatment with hormone replacement therapy or oral anticoagulation.

Prevalence of Val34Leu polymorphism of AF patients was compared to 585 Caucasian, unrelated control subjects from the same geographical area [25]. These control subjects were recruited from blood donors and patients admitted to the traumatology and ophthalmology department who had neither a documented history of vascular disease nor a personal history of thromboembolic or haemorrhagic disease.

Research indices in AF patients were compared to 74 matched controls, in stable sinus rhythm (without any history of AF), of comparable age, sex and co-morbidity, as indicated previously [5]. All subjects were fully informed of the aim of this study. All of them included gave their informed consent to enter in the study, which had been approved by the local Research Committee and was performed in accordance with the Declaration of Helsinki, as amended in Edinburgh in 2000.

2.2. Blood samples and laboratory assays

Venopuncture was performed in the morning on patients, who had been fasting for >12 h and had rested for at least 20 min. Blood samples were obtained by atraumatic venopuncture collection into 1:10 volume of trisodium citrate (Vacutainer, Becton Dickinson, Meylon, France). Platelet poor plasma fractions were obtained by centrifugation at 4 °C for 20 min at 2200 g (within 5 min after blood collection). Aliquots were stored at −30 °C to allow batch analysis. IL6 levels were measured by ELISA with R and D systems reagents (Abingdon, UK), with a minimum sensitivity of 2.5 pg/mL. TF was measured by ELISA (Axis-Shield, UK), with a minimum sensitivity of 10 pg/mL. sPsel was measured by ELISA (Bender MedSystems, Austria), with a minimum sensitivity of 1.3 ng/mL. Finally, fibrinogen levels were determined by the Clauss method in an automated coagulometer (Roche, Switzerland). The intra- and inter-assay coefficients of variation were <5% and 10%, respectively, for all tests.

Total genomic DNA was obtained from the white blood cell fraction after lysis with SDS and proteinase K treatment.
Genomic polymerase chain reaction of the FXIII-A exon 2 gene was performed with mutated primers, essentially as previously described [15].

2.3. Echocardiography

Transthoracic M-mode, two-dimensional and Doppler echocardiography (Hewlett-Packard SONOS 2500, California, USA) were performed in all AF patients. All echocardiographical recordings were performed by the same investigator, and the coefficient of variation for our laboratory was <5%. Echocardiographical measures were performed in the long parasternal and apical four-chamber apical axis (left ventricular end-diastolic and end-systolic diameters, left atrial diameter, ejection fraction, shortening fraction and left ventricular mass index), according to the guidelines laid down by the American Society of Echocardiography.

2.4. Statistical analysis

Continuous variables were tested for normal distribution by Kolmogorov–Smirnov’s test. Data were presented as mean (±S.D., standard deviation) or median (IQR, interquartile range) for continuous variables. The strength of the association of polymorphism and the occurrence of dysrhythmia was estimated by calculation of the odds ratio with EpiInfo software (CDC, Atlanta, Georgia, USA) and the Cornfield method for the calculation of 95% confidence intervals.

Comparisons between two groups were performed by the unpaired t-test, or when appropriated by Mann–Whitney U-test. Categorical data were compared using the Chi-square test, and a Fisher’s exact test was performed if relevant. Correlations between the measured laboratory indices, and clinical, and demographic data were performed using the Pearson correlation coefficient, or Spearman’s test as appropriate and partial correlation coefficients were also calculated, controlling for confounding variables.

Multiple regression analysis (using a stepwise method) was undertaken with the research indices (IL6, TF, fibrinogen and sPSEL) as dependent variables and clinical factors (age, sex, hypertension, diabetes, heart failure, ischaemic heart disease, previous arterial embolism and FXIII Val34Leu polymorphism) and echocardiographical parameters (named: left ventricular end-diastolic and end-systolic diameters, left atrial diameter, ejection fraction, shortening fraction and left ventricular mass index) as independent variables. We included as independent variables in the model those showed a P < 0.150 in the univariate analysis. All analyses were carried out using SPSS version 10.0 software (SPSS Inc. Chicago, IL, USA). A value of <0.05 was considered statistically significant.

3. Results

Clinical characteristics of patients are summarised in Table 1 according to its genotype. The prevalence of heterozygous Val/Leu genotype in 90 patients with persistent AF was 34.4% (31/90), whilst the prevalence of Leu/Leu homozygous subjects was 6.7% (6/90). There were no statistical differences in the FXIII polymorphism prevalence between patients and controls (prevalence of controls: Val/Val 41.1%, Val/Leu 44.4%, Leu/Leu 4.5%, P = 0.236) (Table 2). Patients with AF and Leu34 allele had significantly higher plasma IL6, TF and fibrinogen concentrations (P = 0.004, P = 0.009 and P = 0.023) in AF patients, with no significant differences in plasma fibrinogen concentrations (P = 0.126). The Leu34 allele was associated with higher levels of IL6 (P = 0.004), TF (P = 0.009) and sPSEL (P = 0.023) in AF patients, with no significant differences in plasma fibrinogen concentrations (P = 0.126) (Table 2).

Table 1

<table>
<thead>
<tr>
<th>Val/Val</th>
<th>Val/Leu +</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>53</td>
<td>37 (31 + 6)</td>
<td></td>
</tr>
<tr>
<td>Age (mean ± S.D.)</td>
<td>72.5 ± 8.4</td>
<td>72.6 ± 8.8</td>
</tr>
<tr>
<td>&gt;65 years (%)</td>
<td>47 (88.7)</td>
<td>31 (83.8)</td>
</tr>
<tr>
<td>Male sex (%)</td>
<td>27 (50.9)</td>
<td>17 (45.9)</td>
</tr>
</tbody>
</table>

Data are presented as mean ± S.D.; comparisons by Chi-square test or unpaired t-test.

3.1. The influence of FXIII Val34Leu polymorphism

The Leu34 allele was associated with higher levels of IL6 (P = 0.004), TF (P = 0.009) and sPSEL (P = 0.023) in AF patients, with no significant differences in plasma fibrinogen concentrations (P = 0.126) (Table 2). Patients with AF and Leu34 allele had significantly higher plasma levels of IL6 and TF (P < 0.01), but not sPSEL (P = 0.196) when compared to AF patients with wild genotype and controls. Fibrinogen levels were not significantly different between these groups (Table 2).

3.2. Subgroup analyses

IL6 levels were significantly higher in females (5.5 (2.5–12.6) vs. 3.2 (2.5–8.0) pg/ml; (P = 0.025)), patients with previous embolic event (10.5 (3.3–16.0) vs. 4.0 (2.5–10.0) pg/ml; (P = 0.032)), or with ischaemic heart disease (7.6 (3.1–14.4) vs. 3.6 (2.5–10.0) pg/ml; (P = 0.030)) and in those patients with impaired New York Heart Association (NYHA)
Table 2  
Role of factor XIII Val34Leu polymorphism in research indices in controls and persistent AF patients

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>AF patients</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Val/Val (n = 53)</td>
<td>Val/Leu + Leu/Leu (n = 31 + 6)</td>
<td></td>
</tr>
<tr>
<td>IL6 (pg/ml)</td>
<td>5.0 (2.5–11.0)</td>
<td>3.9 (2.5–8.6)</td>
<td>9.5 (4.0–14.8)</td>
</tr>
<tr>
<td>TF (pg/ml)</td>
<td>21.5 (12.0–48.0)</td>
<td>18.0 (10.8–32.0)</td>
<td>29.5 (17.8–87.5)</td>
</tr>
<tr>
<td>sPsel (ng/ml)</td>
<td>143 (124–177)</td>
<td>180 (150–205)</td>
<td>200 (139–296)</td>
</tr>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>350 ± 85</td>
<td>341 ± 81</td>
<td>363 ± 89</td>
</tr>
</tbody>
</table>

IL6, TF an sPsel comparisons by Mann–Whitney U-test. Fibrinogen comparisons by unpaired t-test.

* IL6 values in Leu allele AF patients vs. controls, P < 0.001.
* TF values in Leu allele AF patients vs. controls P < 0.001.
* sPsel values in Leu allele AF patients vs. controls P = 0.009.
* TF values in AF patients, Val/Val vs. Val/Leu + Leu/Leu, P = 0.004.
* sPsel values in AF patients, Val/Val vs. Val/Leu + Leu/Leu, P = 0.012.

Functional class (8.0 (4.9–11.8) vs. 3.6 (2.5–10.9) pg/ml; (P = 0.024)).

TF levels were higher in females (25.0 (12.0–62.8) vs. 18.5 (10.0–28.0) pg/ml; (P = 0.010)) and in hypertensive patients (25.0 (13.3–49.5) vs. 16.5 (10.0–27.8) pg/ml; (P = 0.010)). sPsel levels were higher in AF patients (183 (151–218) vs. 143 (124–177) ng/ml; (P = 0.003)) and in hypertensive patients (176 (140–202) vs. 142 (124–182) ng/ml; (P = 0.044)). Fibrinogen levels were higher in those subjects >65 years old (363 ± 74 vs. 323 ± 82 mg/dl; (P = 0.020). There were not significant statistical associations between IL6, TF, sPsel or fibrinogen levels, and echocardiographical variables. In addition, all research indices were not significantly affected by aspirin use (data not shown).

3.3. Correlations and multivariate analysis

In the AF patient group, significant correlations were observed between IL6 and TF (Spearman coefficient, r = 0.33; P < 0.01) and fibrinogen levels (Spearman coefficient, r = 0.22; P < 0.05). There were positive correlations between sPsel (Spearman coefficient, r = 0.24; P < 0.01) and fibrinogen levels (Pearson correlation coefficient, r = 0.23; P = 0.032) with age. There were no significant correlations between research indices and the duration of the arrhythmia or echocardiographical parameters (data not shown).

Using a stepwise multiple regression analysis, IL6 levels were independently associated with FXIII Val34Leu polymorphism (P = 0.001), sex (P = 0.031), and previous embolic events (P = 0.005). TF levels was only independently associated with IL6 levels (P = 0.006). The presence of AF was the only variable associated with sPsel levels (P = 0.008). Age was the only variable associated as significant predictor of plasma fibrinogen levels (P = 0.025).

4. Discussion

In the last few years renewed interest has arisen about the importance of abnormalities in haemostasis, platelets and endothelial function in relation to prognosis of AF patients. For example, elevated plasma von Willebrand factor (an index of endothelial damage/dysfunction) was found an independent predictor of cardiovascular events [28]. Recently, raised plasma levels of fibrin D-dimer and tissue plasminogen activator, under oral anticoagulation, were shown to be predictors of cardiovascular events [29].

Several underlying mechanisms have been proposed to promote the prothrombotic state in these patients [30]. Nonetheless, there are limited data on the influence of genetic polymorphisms in the thromboembolic risk associated with AF. In the stroke prevention in atrial fibrillation III study (SPAF III), factor V Leiden was not associated with thromboembolic risk, although there was an independent association with raised levels of prothrombin fragments 1 + 2, a marker of thrombin generation [31]. However, controversial information does exist about the association of the prothrombin G20210A mutation and the occurrence of systemic embolism in AF patients [32,33]. Also, Carter et al. [34] reported that the α-fibrinogen Thr312Ala polymorphism was associated with increased post-stroke mortality in patients with AF. Indeed, this polymorphism seems to influence FXIII cross-linking process, and is suggested to increase susceptibility for embolisation of left appendage clot.

In the present study, FXIII Val34Leu polymorphism was independently associated with the prothrombotic and inflammatory state in AF patients, as evidenced by its association with raised TF and IL6 levels, but not with platelet activation. In the multivariate analysis, only IL6 values were independently associated with the presence of FXIII polymorphism, sex and previous thromboembolism (the two last features being clinical-risk factors for thromboembolism [35]), whereas TF levels were related to IL6 concentrations. These findings confirm recent observations on TF expression, induced by local inflammation, in the endothelium of left atrial appendage may be involved in the pathogenesis of clot formation in AF patients [36].

How would FXIII Val34Leu polymorphism contribute to a pro-inflammatory state in AF? In plasma, FXIII is expressed as a zymogen, whereas in the presence of thrombin and calcium, the A2 unit is released and activated [12]. FXIIIa catalyses the formation of covalent bonds between glutamine and lysine residues in the γ and α chains of adjacent fibrin molecules, which markedly increases the mechanical dura-
bility of the fibrin polymer [37]. The catalytic efficiency of thrombin cleavage of the activation peptide is increased approximately 2.5-fold by the substitution of Val by Leu [18]. Moreover, in situations of relatively lower thrombin concentrations (i.e. in AF, reflected by the continuous and moderately increase of F1+2 prothrombin fragment concentration demonstrated in these patients [5,31]), when FXIII is only partially activated, the differences of FXIII-specific activity between Leu34 and Val34 variants are significantly increased [20]. The nexus between the faster and more efficient activation of FXIII Leu34 and increased IL6 levels might be the expression of FXIII on the surface of monocytes [38]. This would activate a pro-inflammatory response by the monocyte, according to recent studies that have identified a relationship between transglutaminase activity and pro-inflammatory response in other tissues [23].

The data of the present study confirm the existence of an inflammatory state in AF patients [5–7]. Indeed, IL6 levels have also been related to 'low', 'moderate' and 'high'-risk stratification for thromboembolism, using the SPAF-risk stratification criteria, with the highest IL6 levels amongst the high-risk group [39]. Although many questions remained unanswered, the findings of our present study support the hypothesis of the heterogeneity of analysed populations, and clinical characteristics of different disease processes. Hence, the FXIII Val34Leu polymorphism and clinical thromboembolic risk factors were independently associated with IL6 levels in AF. However, the lack of differences in the FXIII Val34Leu polymorphism prevalence between AF patients and controls does not suggest that this polymorphism might promote the development of the arrhythmia. On the contrary, our presented data support the hypothesis that this polymorphism could contribute to thrombogenesis in AF by increasing the inflammatory cytokines.

However, this study is limited by its cross-sectional design. A cross-sectional design allows us only to explore associations, and no causality is implied. Additional studies are necessary to confirm the influence of this polymorphism in the inflammatory state and thrombogenicity in AF, the underlying mechanism, and more importantly its importance in embolic risk.

In conclusion, FXIII Val34Leu polymorphism was independently associated with IL6 levels in AF. The Leu34 allele may potentially influence the prothrombotic state in these patients by modulating the inflammatory state.

References


Lip GYH. The prothrombotic state in atrial fibrillation: the atrium, the endothelium, ... and tissue factor? Thromb Res 2003;111:133–5.


