Thrombosis Research 126 (2010) 159–162

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Regular Article

Factor XIII A subunit Val34Leu polymorphism in patients suffering atherothrombotic ischemic stroke

Amir H. Shemirani [a,b], Endre Pongrácz [c], Bálint Antalfi [d], Róza Ádány [e], László Muszbek [a,b,*]

[a] Clinical Research Center, University of Debrecen, Medical and Health Science Center, Debrecen, Hungary
[b] Thrombosis, Haemostasis and Vascular Biology Research Group of the Hungarian Academy of Sciences, University of Debrecen, Debrecen, Hungary
[c] Department of Neurology, State Health Center, Budapest, Hungary
[d] Department of Pathology, Diósgyőri Vasgyári Hospital, Miskolc, Hungary
[e] Department of Preventive Medicine, University of Debrecen, Medical and Health Science Center, Debrecen, Hungary

ARTICLE INFO

Article history:
Received 9 January 2010
Received in revised form 28 May 2010
Accepted 17 June 2010
Available online 7 July 2010

Keywords:
Cerebrovascular disease
Clinical studies
Factor XIII polymorphism
Gender
Genetic risk factors

ABSTRACT

Introduction: Factor XIII (FXIII) is a key regulator of fibrinolysis and clot firmness. Val34Leu polymorphism of its potentially active A subunit (FXIII-A) leads to faster activation of FXIII, influences clot structure and provides a moderate protection against coronary artery disease. The effect of FXIII A subunit (FXIII-A) Val34Leu polymorphism on the risk of ischemic stroke (IS) has been investigated in a few studies with contradictory results. In spite of their fundamental difference in pathogenesis and hemostatic pathomechanism, only four small studies investigated the effect of FXIII-A Val34Leu polymorphism on the risk of atherothrombotic IS (AIS) separately from cardioembolic IS. Gender specific effect of the polymorphism on the risk of AIS has not been explored. In the present study we investigated the effect of FXIII-A Val34Leu polymorphism on the risk of AIS on a large patient population.
Materials and methods: A population control group of 1,146 randomly selected individuals, 496 patients surviving AIS and their age and sex-matched controls selected from the population control group were included in the study. FXIII-A Val34Leu genotype was determined on DNA samples, obtained from peripheral blood leukocytes, by fluorescence resonance energy transfer detection using melting curve analysis.
Results: Neither sex nor age affected the distribution of FXIII-A Val34Leu genotypes in population control group. No association was revealed between the risk of AIS and FXIII-A Leu34 carriership and homozygous or heterozygous presentation of Leu34 allele in either gender.
Conclusion: FXIII-A Val34Leu polymorphism fails to influence the risk of AIS.

Introduction

Blood coagulation factor XIII (FXIII) is a tetrameric zymogen consisting of two potentially active A subunits (FXIII-A) and two carrier/inhibitory B subunits (FXIII-B) [1,2]. It is activated by the concerted action of thrombin and Ca^{2+}. Thrombin cleaves off a peptide of 37 amino acids from the N-terminal end of FXIII-A, and then in the presence of Ca^{2+} FXIII-B dissociates and the dimer of FXIII-A becomes an active transglutaminase (FXIIIa) that cross-links peptide chains by isopeptide bonds. FXIIIa cross-links fibrin γ-chains into dimers and fibrin α-chains into high molecular weight polymers. This way it strengthens fibrin clots and makes them more resistant against shear stresses. FXIIIa also attaches α_2 plasmin inhibitor to fibrin and protects newly formed fibrin from the prompt elimination by the fibrinolytic enzyme plasmin. Thrombin activable fibrinolysis inhibitor, plasminogen activator inhibitor 2 and plasminogen are also substrates of FXIIIa, which is now considered as one of the key regulators of fibrinolysis [3].

In the coding region of FXIII-A gene there are five single nucleotide changes that give rise to the following common polymorphisms in the amino acid sequence: Val34Leu, Tyr204Phe, Leu564Pro, Val650Ile and Glu651Gln. FXIII-A Val34Leu polymorphism [4] has stirred considerable interest in the last decade. Its prevalence is about 25% among Caucasians, while in blacks and Asian Indians the allele frequency is lower, and in the Japanese population it is extremely rare (www.hapmap.org and reference [5]). In 1998 it was demonstrated in a pioneering case-control study that the presence of FXIII-A Leu34 allele provided protection against myocardial infarction (MI) [6]. Since then, several confirmatory and contradictory reports have been published on the association of FXIII-A Val34Leu polymorphism and coronary artery disease (CAD) (see reviewed in reference [7]), and meta-analysis of published results concluded that carriership of the Leu34 allele provided a moderate (odds ratio 0.81, 95% CI 07-0.92), but

Abbreviations: FXIII, Blood coagulation factor XIII; FXIII-A, F-XIII A subunit; FXIII-B, F-XIII B subunit; MI, myocardial infarction; CAD, coronary artery disease; IS, ischemic stroke; AIS, atherothrombotic IS.

* Corresponding author. Clinical Research Center, Medical and Health Science Center, University of Debrecen, PO Box 40, Debrecen H-4012, Hungary. Tel.: +36 52 431956; fax: +36 52 340011.
E-mail address: muszbek@med.unideb.hu (L. Muszbek).

0049-3848/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.thromres.2010.06.012

Table 1
The distribution of FXIII-A Val34Leu genotypes in the population control group.

	Total	Gender		Age distribution		
		Female	Male	1st tertile	2nd tertile	3rd tertile
Number	1146	614	532	382	382	382
Age: median (IQR)	46 (33-60)	48 (34-63)	44 (31-57)	28 (24-33)	46 (42-50)	66 (60-74)
Val34Leu genotype						
Val/Val	629 (54.9%)	342 (55.7%)	287 (53.9%)	205 (53.7%)	212 (55.5%)	212 (55.5%)
Val/Leu	440 (38.4%)	237 (38.6%)	203 (38.2%)	147 (38.5%)	141 (36.9%)	152 (39.8%)
Leu/Leu	77 (6.7%)	35 (5.7%)	42 (7.9%)	30 (7.9%)	29 (7.6%)	18 (4.7%)
L34 carriers	517 (45.1%)	272 (44.3%)	245 (46.1%)	177 (46.3%)	170 (44.5%)	170 (44.5%)
L34 allele	25.9%	25.0%	27.0%	27.0%	26.0%	24.6%

IQR, inter-quartile range.

statistically significant protection against CAD or MI [8]. The association of FXIII-A Val34Leu polymorphism with ischemic stroke (IS) has also been addressed in a few studies with contradictory results [9–20]. Although the development of atherothrombotic IS (AIS) and cardioembolic IS involve different pathogenesis and hemostatic pathomechanisms, in the majority of these studies the two types of stroke were not separated. In these previous studies gender specific effects were not explored. In the present case-control study the prevalence of FXIII-A Val34Leu polymorphism was investigated in male and female survivors of AIS and it was compared to its prevalence in age and sex matched and non-matched population control groups.

Materials and methods

Selection of cases and controls

508 consecutive patients who survived IS were included in the study, no one refused. The diagnosis of IS was based on clear, unambiguous clinical symptoms persisting for more than 24 hours, and was confirmed by computed tomography or nuclear magnetic resonance imaging. In 12 patients cardioembolic IS was diagnosed; they were excluded from the study and only patients with AIS remained. It is to be noted that the patients were enrolled at a military hospital and they had a relatively younger age and male dominance.

The population control group that represented the general Hungarian population consisted of 1,146 Hungarian individuals [21]. The sampling frame for the reference group included all those registered with the participating practices in the Hungarian General Practitioners' Morbidity Sentinel Stations Program. 22 practitioners were selected from four counties in a way to represent the distribution of settlement size of each county and thereafter were asked to invite individuals randomly according to a previously specified algorithm from their practices. We selected age and sex matched controls to the patient groups from this population control group.

Ethical approval for the study was obtained from the Ethics Committee of the Medical and Health Science Center, University of Debrecen, Hungary. Individuals in the population control group and patients surviving AIS gave informed consent.

Laboratory Methods

DNA from controls and from patients surviving AIS was isolated from the buffy coat of citrated blood samples by QIAamp DNA Blood Mini Kit (Qiagen). Val34Leu polymorphism was determined by real time PCR using fluorescence resonance energy transfer detection and melting curve analysis on LightCycler equipment (Roche Diagnostics) according to the method developed in our laboratory [22].

Statistical analysis

χ^2 test was used for differences in category frequencies. A p-value of 0.05 or less was considered to indicate statistical significance. The effect of FXIII-A genotype was expressed as the odds ratio (OR) and 95% confidence interval (CI), which were computed from the corresponding regression coefficient in the logistic regression model. In comparisons with the total population control group adjusted ORs were obtained by the use of a model that included FXIII-A genotype, age and gender. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS 13.0).

Results

Gender-, and age-dependent distribution of FXIII-A Val34Leu genotype in the general population

As we intended to investigate gender specific differences in the effect of FXIII-A Val34Leu genotypes on the risk of AIS, first we explored the frequency of these genotypes in a population control group that represent the general Hungarian population. There was no gender-specific difference in the frequency of FXIII-A Val34Leu genotypes, Leu34 carriers and Leu34 allele in the population control group (Table 1). The effect of age on the distribution of Val34Leu genotype was investigated by dividing the population control group into age tertiles and comparing them to each other. The lack of statistically significant difference among the age groups indicates that in the general population FXIII-A Val34Leu polymorphism does not provide survival advantage or disadvantage.

General charcteristics of the patient population

Table 2 demonstrates the general characteristics of patients involved in the study. In this patient group the median age of males suffering AIS was somewhat higher than that of females and hyperlipidemia (cholesterol >5.2 mmol/L and/or triglyceride >1.7 mmol/L) was more frequent in male than in female patients. There was no gender difference in the frequency of diabetes mellitus and hypertension.

Table 2
General characteristics of study population with non-fatal atherothrombotic ischemic stroke (AIS).

	Patients surviving AIS		
	Total	Female	Male
Number	496	159	337
Age: median (IQR)	51 (44-62)	47 (41-59)	53 (45-63)*
Hypertension	140 (28.2%)	39 (24.5%)	101 (30.0%)
Hyperlipidemia	119 (24.0%)	21 (13.2%)	98 (29.1%)*
Diabetes mellitus	41 (8.3%)	11 (6.9%)	30 (8.9%)
Oral contraceptives	21 (4.2%)	21 (13.2%)	n.a.

IQR, inter-quartile range. *$p<0.001$.