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Full Length Article

Regulation of plasma factor XIII levels in healthy individuals; a major impact by subunit B intron K c.1952 + 144 C>G polymorphism

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ABSTRACT

Background: The regulation of plasma factor XIII (FXIII) levels in healthy individuals has been only partially explored. The identification of major non-genetic and genetic regulatory factors might provide important information on the contribution of FXIII to the risk of cardio/cerebrovascular diseases.
Objectives: To determine the effect of age, smoking, BMI, fibrinogen concentration on plasma FXIII activity, complex FXIII antigen (FXIII-A_2B_2) and total FXIII-B subunit (tFXIII-B) level, to correlate FXIII-B level with the other two FXIII parameters and to assess the variation of FXIII levels in carriers of major FXIII subunit polymorphisms.
Methods: 268 healthy individuals were enrolled in the study. FXIII activity was measured by the ammonia release assay; FXIII-A_2B_2 and tFXIII-B were determined by ELISAs. FXIII-A p.Val34Leu, FXIII-B p.His95Arg and FXIII-B intron K c.1952 + 144 C>G polymorphisms were identified by RT-PCR using melting point analysis with fluorescence resonance energy transfer detection.
Results: All investigated FXIII parameters showed significant positive correlation with age and fibrinogen level; gender and BMI influenced only tFXIII-B. A highly significant positive correlation was demonstrated between tFXIII-B and the other FXIII parameters. FXIII-A p.Val34Leu polymorphism had only slight, if any effect on FXIII levels. The FXIII-B Arg95 allele moderately increased all three FXIII parameters, but the effect became statistically significant only after adjustment. The FXIII-B intron K G allele drastically decreased FXIII levels, and it seemed to be in synergism with the FXIII-A Leu34 allele.
Conclusions: Plasma FXIII levels are subjected to multifactorial regulation, in which age, fibrinogen level and FXIII-B intron K polymorphism are major determinants.

1. Introduction

Blood coagulation factor XIII (FXIII) circulates in the plasma as heterotetramer of two catalytic A subunits (FXIII-A) and two carrier/inhibitory B subunits (FXIII-B). The zymogen FXIII is activated by thrombin and Ca^{2+}; thrombin cleaves off an activation peptide of 37 amino acids from the N-terminus of FXIII-A, then in the presence of Ca^{2+} the two subunits dissociate and FXIII-A assumes an enzymatically active configuration (FXIIIa). FXIIIa is a transglutaminase the main task of which is to cross-link fibrin γ-, and α-chains and α_2-plasmin inhibitor to fibrin. This way it protects newly formed fibrin from the shear stress of circulating blood and from degradation by the fibrinolytic enzyme, plasmin. FXIII-B significantly prolongs the lifespan of FXIII-A in the circulation and prevents its spontaneous progressive activation. FXIII-A is synthesized by cells of bone marrow origin, while FXIII-B is produced by hepatocytes. FXIII-B is in excess to FXIII-A, in plasma approximately 50% of it exists in free, non-complexed form. Both subunits are polymorphic; the major polymorphisms in the Caucasian population are p.Val34Leu (rs5985) in FXIII-A, p.His95Arg (rs6003) and IVS11, c.1952 + 144 C>G (rs12134960) in FXIII-B. The latter polymorphism introduces a new splice site in intron K, which results in the replacement of the last 10 C-terminal amino acids by 25 new amino acids. (For further details on the structure and function of FXIII references [1–4] should be consulted.)

Plasma FXIII levels are regulated by both genetic and non-genetic factors [5–7]. A single study evaluated the effect of age, gender, smoking and hypertension on FXIII subunit and activity levels in elderly healthy

Abbreviations: BMI, body mass index; FXIII, plasma factor XIII; FXIIIa, activated FXIII; FXIII-A, FXIII A subunit; FXIII-B, FXIII B subunit; tFXIII-B, total FXIII-B subunit.

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individuals (age: 63.8 ± 16.8 years) [7]. Using likelihood analysis with adjustment for age and gender 47% hereditability was established for the plasma level of complex FXIII (FXIII-A_2B_2) in healthy families [5]. The common FXIII-A polymorphism, p.Val34Leu accounted only for a small fraction of this hereditability. The common FXIII-B polymorphisms were discovered later and evidently were not included in this study. Most recent studies indicate that FXIII-B polymorphisms might be important factors in assessing the risk of myocardial infarction and stroke [8,9]. In a study concerning the risk of venous thrombosis no significant effect of FXIII-B p.His95Arg polymorphism on FXIII activity, FXIII-A, FXIII-B and FXIII-A_2B_2 was revealed in a combined group of controls and patients with vascular disease [10]. In another study FXIII-B Arg95 allele slightly but significantly increased FXIII activity and antigen level in a merged group of clinical controls and patients with coronary artery disease [8]. The recently discovered FXIII-B polymorphism in intron K of the FXIII-B gene (nt29756, c.1952 + 144 C>G) [11,12] decreased FXIII levels both in clinical controls and in cardiovascular patients [8]. Neither study investigated the effect of FXIII-B polymorphisms on plasma FXIII levels in healthy individuals. Here we explored the effect of age, body mass index (BMI), smoking and fibrinogen level on FXIII activity, FXIII-A_2B_2 and FXIII-B antigen levels in males and females. The correlation between the three FXIII parameters was also investigated. Finally, we determined how three FXIII polymorphisms, FXIII-A p.Val34Leu, FXIII-B p.His95Arg, FXIII-B intron K C>G and their combinations influence FXIII levels.

2. Methods

2.1. Study population

268 apparently healthy young and middle-aged adults from Eastern Hungary were enrolled in the study. All individuals were informed about the study according to the study protocol, and gave written informed consent. Ethical approval for the study was obtained from the Ethics Committee of the Medical Faculty, University of Debrecen, Hungary. The characteristic features of the study population are shown in Table 1. 53 individuals had moderate hypertension (between 145/90 and 165/95 Hgmm) and were on antihypertensive therapy, which was not considered as exclusion criteria. Among the 160 women 19 were taking oral contraceptive and 36 were in menopause. The BMI were calculated and smoking habit was recorded.

Table 1
Characteristics of study population; comparison of females and males.

	Total (n = 268)	Female (n = 160)	Male (n = 108)	Significance (p)
Age	38.0 (26.0–48.0)	39.0 (24.3–48.0)	36.5 (27.0–46.0)	0.726
BMI	24.9 (21.5–28.4)	22.7 (20.3–26)	26.7 (24.2–29.2)	<0.001
Smoking (−/+)	200/68	123/37	77/31	0.319
Fibrinogen (g L^{-1})	3.4 ± 0.6	3.5 ± 0.6	3.2 ± 0.6	0.015
FXIII activity (%)				
Non-adjusted	108.4 ± 25.0	110.1 ± 23.7	105.9 ± 26.6	0.175
Adjusted		109.1 ± 23.7	107.3 ± 26.6	0.470
FXIII-A_2B_2 antigen (%)				
Non-adjusted	105.9 ± 23.8	107.2 ± 22.2	103.9 ± 26.0	0.262
Adjusted		106.4 ± 22.2	105.2 ± 26.0	0.646
FXIII-B (%)				
Non-adjusted	108.5 ± 18.0	105.8 ± 18.2	112.4 ± 16.9	0.003
Adjusted		106.0 ± 18.2	112.1 ± 16.9	0.003
FXIII-A p.Val34Leu				
Wild type	133	82	51	
Heterozygote	106	60	46	
Homozygote	29	18	11	
Leu34 carrier	50.4%	48.8%	52.8%	0.536
Leu34 allele frequency	30.6%	30.0%	31.5%	0.878
FXIII-B p.His95Arg				
Wild type	216	128	88	
Heterozygote	47	31	16	
Homozygote	5	1	4	
Arg95 carrier	19.4%	20.0%	18.5%	0.875
Arg95 allele frequency	10.6%	10.3%	11.1%	0.817
FXIII-B intron K C > G				
Wild type	203	115	88	
Heterozygote	60	42	18	
Homozygote	5	3	2	
G carrier	24.3%	28.1%	18.5%	0.082
G allele frequency	13.1%	15.0%	10.2%	0.285

Parameters showing non-parametric distribution (age and BMI) are represented by median and interquartile range, while in the case of other parameters with parametric distribution mean ± SD are shown. Smoking - and + represent non-smokers and current smokers, respectively. Significance concerns differences between females and males. FXIII activity and FXIII-A_2B_2 antigen were adjusted to age and fibrinogen concentration. FXIII-B antigen was adjusted to age, BMI and fibrinogen concentration.

2.2. Laboratory methods

Fasting blood samples were collected from the antecubital vein into 1/10 volume of 0.109 M citrate. Platelet poor plasma was separated by centrifugation at 2500*g* for 20 min, and aliquots were stored at −70 °C until measurements. DNA was isolated from the buffy coat of citrated blood samples by QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). FXIII activity was measured by ammonia release assay [13] using REA-chrom FXIII kit (Renal-ker, Budapest, Hungary). FXIII-A_2B_2 and total FXIII-B antigen concentrations were determined by sandwich ELISA [14,15]. FXIII activity and subunit levels in all subgroups showed normal distribution and they were expressed as percentages of pooled normal plasma. The Clauss method was used for the measurement of fibrinogen concentration. FXIII-A p.Val34Leu, FXIII-B p.His95Arg and FXIII-B Intron K c.1952 + 144 C>G polymorphisms were determined according to protocols developed in our laboratory [8,16].

2.3. Statistical analysis

The distribution of parameters was examined by the Kolmogorov-Smirnov and Shapiro-Wilk tests. Results of parametric variables were expressed as mean ± SD, while results of non-parametric variables were shown as median and interquartile range. Between group differences were analyzed by Student's t test when normally distributed and by Mann-Whitney test when the distribution was non-parametric. Differences in category frequencies were evaluated by χ^2 test. Pearson's correlation coefficient was calculated to characterize the strength of the linear relationship between two variables. Multiple linear regression analysis was performed to adjust for parameters independently associated with FXIII levels. The significance of differences in mean FXIII levels was tested by analysis of variance (ANOVA) using the Bonferroni correction for multiple comparisons. A p value of <0.05 was considered as statistically significant. The Statistical Package for the Social Sciences (SPSS 22, Chicago IL) was used for statistical analyses.

3. Results and discussion

3.1. Characterization of study population

Table 1 demonstrates the gender specific differences in the investigated parameters. Males and females were of similar age; there were more current smokers among males (29%) than among females (22%), but the difference was not statistically significant. Males had considerably higher BMI and somewhat lower fibrinogen level than females. In our population neither FXIII-A_2B_2 antigen level nor FXIII activity showed gender specific differences, while total FXIII-B concentration was significantly higher in males than in females. Ariens et al. reported higher FXIII-A antigen concentration in females and no difference in FXIII