

Factor XIII Val34Leu polymorphism as a modulator of fibrin clot permeability and resistance to lysis in patients with severe coronary artery disease

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Abstract

Background: A common G to T transition in codon 34 with the subsequent valine with leucine replacement in the factor (F) XIII A-subunit affects fibrin formation and stabilisation in vitro. Data on the effects of Leu34 allele on cardiovascular thromboembolic events in vivo are conflicting.

Aim: We investigated whether FXIII Val34Leu polymorphism is potent enough to affect fibrin clot properties in patients with advanced coronary artery disease (CAD).

Methods: We studied 113 patients, aged 62.8 ± 6.1 years, who were scheduled for elective isolated coronary artery bypass grafting surgery (CABG). Patients were compared with 98 healthy age-matched controls. Ex vivo fibrin clot permeability and lysis time ($t_{50\%}$) were determined in citrated plasma.

Results: Patients scheduled for CABG had lower clot permeability (9.14 ± 1.64 vs. $10.02 \pm 1.12 \times 10^{-9}$ cm²; $p = 0.0002$) and longer $t_{50\%}$ (8.45 ± 1.94 vs. 7.63 ± 1.24 min; $p < 0.0001$) than controls. The Leu34 carriers, i.e. 9 (8%) Leu34Leu homozygous and 23 (20%) Val34Leu heterozygous subjects, had lower permeability by 23% in the CAD group compared with 81 (72%) patients with Val34Val genotype. A similar intergroup difference was observed for $t_{50\%}$ which was longer in Leu34 carriers ($p < 0.0001$). The FXIII Leu34 allele frequency in the control group was similar as well as the impact of Leu34 allele on fibrin properties. The effect of FXIII Leu34 allele on permeability and $t_{50\%}$ was not affected by homocysteine, C-reactive protein and fibrinogen levels in CAD patients and controls.

Conclusions: Like in healthy subjects, in patients scheduled for CABG, the FXIII Leu34 allele is associated with decreased fibrin clot permeability and efficiency of lysis.

Key words: factor XIII, fibrinolysis, coronary artery disease, polymorphism

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Introduction

Factor XIII (FXIII) is a thrombin-activated transglutaminase that forms covalent bonds between gamma-chains and between gamma- and alpha-chains of fibrin fibers to form gamma-chain dimers and high-molecular weight alpha-chain polymers. An activated FXIII (FXIIIa) stabilises fibrin clots by cross-linking fibrin molecules and other proteins including fibronectin and collagen [1]. The covalent fibrin cross-linking by FXIIIa leads to the formation of a firm and an elastic structure of fibrin gel and the reduction in clot susceptibility to proteolytic or mechanical degradation [1]. Clot stabilisation is regulated

by the FXIIIa cross-linking between alpha2-antiplasmin to the fibrin alpha-chains and plays a major role in the modulation of fibrinolysis [2].

A common coding polymorphism in the FXIII gene, a G100T substitution in exon 2 of the A-subunit, which causes an amino acid transition at codon 34 (Val34Leu) has been reported to affect fibrin formation and stabilisation [3]. The Val34Leu polymorphism does not influence the transglutaminase activity of FXIIIa, but results in earlier FXIII activation and eventually accelerates the cross-linking of fibrin gamma- and alpha-chains [3]. It seems that early fibrin cross-linking in the presence

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of FXIII^{Leu34} variant inhibits lateral aggregation of fibrin fibers, whereas delayed cross-linking in the Val34 variant provides more lateral aggregation [4]. More rapidly activated Leu34 FXIII produces a fibrin meshwork with thin fibers and small pores [4]. The FXIII^{Leu34} allele is associated with decreased fibrin clot permeation [5, 6]. Moreover, clot formation time measured by thromboelastography is significantly shortened in the FXIII^{Leu34} positive samples [7].

A few years ago several clinical trials indicated that the Leu34 FXIII allele is associated with a reduced risk of myocardial infarction (MI) [8], deep vein thrombosis [9], stroke due to small vessel disease and intracerebral hemorrhage [10, 11]. However, more recent studies yielded conflicting results suggesting that the Leu34 allele has neutral or harmful effects in the same clinical settings [12, 13]. Meta-analysis published by Shafey et al. [13] demonstrated that FXIII^{Leu34} allelic variant is associated with higher myocardial infarction rates. These differences have been attributed to geographical locations, ethnicity and patient characteristics.

Some confounders have been identified to influence results of clinical studies on FXIII. It has been suggested that aspirin administration modulates the impact of the Leu34 allele on FXIII activation and cross-link formation *via* fibrin (ogen) acetylation resulting in slower FXIII activation in the Leu34 carriers on aspirin [14]. On the other hand, several *in vitro* and *ex vivo* studies demonstrated that a number of other variables can alter the fibrin structure such as the concentration and function of fibrinogen [15] or thrombin [16], levels of homocysteine [17], lipoprotein (a) [18], or C-reactive protein (CRP) [19]. The impact of the last 3 latter modulators in patients with advanced coronary artery disease (CAD) has been reported by our group recently [20]. Lim et al. showed that increased fibrinogen concentrations are associated with decreased clot permeability and tighter clot structure in the presence of FXIII^{Val34} allele compared with those in the presence of the Leu34 allele [6]. Although genetic polymorphisms affect the clot permeability and largely contribute to variability of clot properties and resistance to lysis, their actual impact on fibrin clot function and architecture evaluated using plasma-based assays remains unclear. It is also unclear whether plasma-related factors alter fibrin clot features irrespective of FXIII^{Val34}Leu polymorphism.

Previous studies on FXIII^{Val34}Leu polymorphism were performed in models based on purified fibrinogen. This approach abolishes the effect of other plasma components known to affect the clot architecture and properties [16-19]. To our knowledge, there have been no larger studies on the role of FXIII^{Leu34} allele in patients with severe CAD in relation to clot properties assessed in plasma-based assays.

We sought to investigate the impact of the FXIII gene Val34Leu polymorphism on two crucial fibrin clot features,

fibrin clot permeability and fibrinolysis, in patients with severe CAD scheduled for coronary artery bypass grafting surgery (CABG).

Methods

Patients

One hundred thirteen consecutive patients with angiographically proven two- or three-vessel CAD (at least 50% stenosis in a major epicardial artery), who were scheduled for elective isolated CABG, were recruited from the Department of Cardiovascular Surgery and Transplantology, Institute of Cardiology, Jagiellonian University School of Medicine. Exclusion criteria were: any acute illness; cancer; hepatic (alanine aminotransferase > 1.5 times above the upper limit) or renal dysfunction (creatinine > 177 µmol/l); anticoagulant therapy; acute coronary syndrome within the previous 6 weeks; a history of venous thromboembolism. All medications were administered in the unchanged doses for at least 2 previous weeks, except aspirin which was discontinued in some patients (8%) no longer than 3 days prior a surgery. Diabetes was defined as fasting glucose above 7 mmol/l or treatment with hypoglycemic agents. Hypertension was defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg and/or use of antihypertensive agents. Subjects who smoked regularly were classified as current smokers. The body mass index (BMI) was calculated as follows: body weight (kg)/height (m²). Previous MI was diagnosed based on medical records. Peripheral artery occlusive disease (PAOD) was diagnosed in subjects with intermittent claudication (class II according to Fontaine). Ninety-six apparently healthy individuals matched for age and sex were recruited from the hospital staff served as controls. They were free from a personal and family history of CAD or venous thromboembolism. The University Ethical Committee approved the study, and patients provided written, informed consent.

Blood collection

Venous blood samples were drawn between 6 and 10 AM after an overnight fast. Blood was taken into 0.13 trisodium citrate tubes (Becton Dickinson) and centrifuged within 30 min at room temperature at 2560 g for 20 min. Platelet-poor plasma was frozen in aliquots at -80°C until further use. Blood samples for total homocysteine (Hcy) determination were taken into EDTA tubes and placed directly on ice until the plasma was separated.

Laboratory investigations

Lipid profiles, blood cell counts, glucose, and creatinine were assayed by routine laboratory techniques. Fibrinogen and high-sensitivity CRP were measured by nephelometry (Dade Behring). Fasting plasma Hcy levels were measured

using an immunoassay (IMX System, Abbott Diagnostics). Lipoprotein (a) levels were measured using an ELISA (Biopool, Sweden).

Fibrin permeability

Ex vivo fibrin clot permeability was determined as previously described [21]. Briefly, 20 mmol/L calcium chloride and 1 U/mL human thrombin (Sigma) were added to 120 μ l citrated plasma samples. After incubation in a wet chamber for 120 min, tubes containing the clots were connected to a reservoir of a buffer (0.05 M Tris HCl, 0.15 M NaCl, pH 7.5) and its volume flowing through the gels was measured. A permeation coefficient, or Darcy constant (K_s), which indicates the pore size, was calculated from the equation: $K_s = Q \times L \times \eta / t \times A \times \Delta p$, where Q is the flow rate in time t , L is the length of a fibrin gel (13 mm), η is the viscosity of liquid (1/100 poise), A is the cross-sectional area (0.049 cm²), and Δp is a differential pressure (in dyne/cm²). All measurements were performed in duplicates by an investigator blinded to the origin of the plasma studied. The intraindividual variability of results was ~7%.

Clot lysis assay

Clot lysis times were determined by measuring turbidity profiles of a plasma fibrin clot as a function of time using a method by Williams et al. [22] with our slight modifications [21]. Citrated plasma samples (100 μ l) was diluted 1 : 1 with a buffer (0.05 M Tris HCl, 0.15 M NaCl, pH 7.4), containing 20 mmol/l calcium chloride, 1 IU/ml human thrombin (Sigma) and 1 μ g/ml recombinant tissue plasminogen activator, rtPA (Boehringer Ingelheim). Assembly kinetics were monitored by spectrophotometry at 405 nm in duplicate aliquots in a Spectramax 340 kinetic microplate reader (Molecular Devices Corp., Menlo Park, CA). The time required for a 50% decrease in clot turbidity (time to half-lysis, $t_{50\%}$) was chosen as a marker of the clot susceptibility to fibrinolysis. The intra-assay and interassay coefficients of variation for all turbidity measures were between 4 to 7%.

Genotyping

The FXIII Val34Leu polymorphism was performed on an ABI PRISM® 7900HT Fast Real-Time PCR System. The assay was ordered from Applied Biosystems as Pre-Designed/Validated Assays for a human factor XIII (rs5985), a context sequence ([VIC/FAM]): ACCTGCC CACAGTGGAGCTTCAGGGC [G/T] TGGTGGCCCGGGCGTCA ACCTGCA.

Statistical analysis

Hardy-Weinberg equilibrium was used to calculate the expected genotype distribution under equilibrium assumptions. The observed and expected numbers of each

genotype were compared by χ^2 test (OEGE online tools; <http://www.oege.org/software/>). Results are shown as mean \pm standard deviation (SD). The Kolmogorov-Smirnov one-sample test was performed for testing the sample cumulative distribution. The Pearson's correlation coefficient was determined for the two variables regression analysis. Comparisons between 2 groups were performed by the Student's t-tests. Categorical values were analysed using the χ^2 test. Factorial ANOVA was performed to analyze non-interactive effects of Val34Leu genotype and the fibrin parameters. A p-value \leq 0.05 was considered statistically significant.

Results

Characteristics of the studied groups

The CAD group comprised 113 patients aged 62.8 ± 6.1 years, including 63 men (71%). Forty-five (40%) patients were active smokers and 24 (21%) subjects had type 2 diabetes (Table I). Ninety-two percent of patients took low-dose aspirin on a regular basis, while 88% were treated with statins. Patients with severe CAD and controls did not differ with respect to age, gender, BMI, smoking status, lipid profile, glucose and creatinine (Table I). CAD patients had significantly higher fibrinogen, CRP, Hcy, and Lp (a) than controls (Table I).

While comparing the patient and control groups, we found that patients scheduled for CABG had lower clot permeability (K_s) (9.14 ± 1.64 vs. $10.02 \pm 1.12 \times 10^{-9}$ cm²; $p = 0.0002$) and longer lysis time ($t_{50\%}$) (8.45 ± 1.94 vs. 7.63 ± 1.24 min; $p < 0.0001$). In both groups there were strong correlations between age and both K_s ($r = -0.82$; $p < 0.001$ for controls and $r = -0.63$; $p < 0.0001$ for CAD patients) and $t_{50\%}$ ($r = 0.72$; $p < 0.0001$ and $r = 0.51$; $p < 0.0001$, respectively). Diabetic patients tended to display 15% lower K_s ($p = 0.07$) and 12% longer $t_{50\%}$ ($p = 0.09$) compared to nondiabetic subjects. There were no significant differences between current smokers and the remaining patients ($p > 0.2$ for both variables). This held true also for subjects with previous MI, revascularisation or hypertension (data not shown). Given relatively small numbers of patients not receiving aspirin or statins, a potential effect of both medications was also undetectable.

The prevalence of the FXIII Val34Leu polymorphism in CAD group deviated from Hardy-Weinberg equilibrium ($p < 0.001$). The allele frequency was as follows: 81 (72%) patients were homozygous for the Val34 allele, 9 (8%) were homozygous for the Leu34 allele and 23 (20%) were heterozygous for the Leu34 allele. The allele frequency in the control group was similar, 68 (71%), 8 (8%) and 20 (21%), respectively.

Carriers of the Leu34 allele and the Val34 homozygotes did not differ with regard to clinical and demographic variables except age; the Leu34 carriers were older in both groups than non-carriers (68.1 ± 5.7 vs. 58.5 ± 3.5 years for

Table I. Characteristics of patients with advanced coronary artery disease (CAD) and apparently healthy controls

Variable	CAD patients (n = 113)	Controls (n = 96)	P
Age [years]	62.8 ± 6.1	61.3 ± 6.1	NS
Male gender, n (%)	63 (71)	77 (74)	NS
BMI [kg/m ²]	26.8 ± 2.9	26.3 ± 3.6	NS
Current smokers, n (%)	45 (40)	43 (45)	NS
Arterial hypertension, n (%)	59 (52)	0 (0)	n/a
Diabetes, n (%)	24 (21)	0 (0)	n/a
Previous MI, n (%)	67 (59)	0 (0)	n/a
Previous revascularisation, n (%)	25 (22)	0 (0)	n/a
PAOD, n (%)	20 (18)	0 (0)	n/a
TC [mmol/l]	5.84 ± 1.12	5.69 ± 0.87	NS
LDL-C [mmol/l]	3.99 ± 0.98	3.92 ± 0.71	NS
HDL-C [mmol/l]	1.18 ± 0.27	1.22 ± 0.29	NS
TG [mmol/l]	1.82 ± 0.81	1.63 ± 0.9	NS
Glucose [mmol/l]	5.7 ± 0.6	5.4 ± 0.5	NS
Creatinine [μmol/l]	88.5 ± 10.2	84.9 ± 8.3	NS
Fbg [g/l]	2.89 ± 0.72	2.68 ± 0.51	0.0008
CRP [mg/l]	1.86 ± 0.76	1.63 ± 0.63	0.05
Hcy [μmol/l]	13.38 ± 4.84	11.80 ± 2.84	< 0.0001
Lp(a) [mg/dl]	18.96 ± 9.81	12.18 ± 4.45	< 0.0001
Aspirin, n (%)	104 (92)	0	n/a
Statin, n (%)	99 (88)	0	n/a
Beta-blockers, n (%)	53 (47)	0	n/a
ACE inhibitors, n (%)	49 (43)	0	n/a
Calcium antagonists, n (%)	30 (27)	0	n/a
Diuretics, n (%)	41 (36)	0	n/a
Nitrates, n (%)	83 (73)	0	n/a
Fibrates, n (%)	17 (15)	0	n/a

Data are given as mean ± SD, or number (percentage) as appropriate; n/a – not applicable

Abbreviations: ACE – angiotensin-converting enzyme, BMI – body mass index, CRP – C-reactive protein, Fbg – fibrinogen, Hcy – total homocysteine, HDL-C – high density lipoprotein cholesterol, LDL-C – low density lipoprotein cholesterol, MI – myocardial infarction, PAOD – peripheral arterial occlusive disease, TC – total cholesterol, TG – triglycerides, NS – non-significant

CAD patients and 67.8 ± 5.7 vs. 60.1 ± 5.2 years for controls; $p < 0.0001$, respectively).

The Leu34 carriers had lower permeability in the CAD group (7.29 ± 1.90 vs. $9.86 \pm 1.70 \times 10^{-9}$ cm², $p < 0.0001$, respectively) and in the controls (8.65 ± 0.15 vs. $10.58 \pm 0.80 \times 10^{-9}$ cm², $p < 0.0001$, respectively) as compared to those homozygous for Val34 allele. Similar intergroup differences were observed for lysis time which was longer in subjects possessing FXIII Leu34 allele compared to the non-carriers (9.96 ± 3.35 vs. 7.86 ± 1.50 min for CAD patients, $p < 0.0001$, and 8.93 ± 0.26 vs. 7.10 ± 0.60 min for controls, $p < 0.0001$, respectively) (Table II). Interestingly, levels of Hcy, Lp(a), CRP and fibrinogen were significantly higher in Leu34 carriers than in Val34Val subjects in both groups (Table II).

Modulators of the clot structure

Patients and controls were stratified according to fibrinogen, Hcy, and CRP. The CAD group and controls were divided into terciles and factorial ANOVA analysis in respect of the FXIII Val34Leu polymorphism was performed.

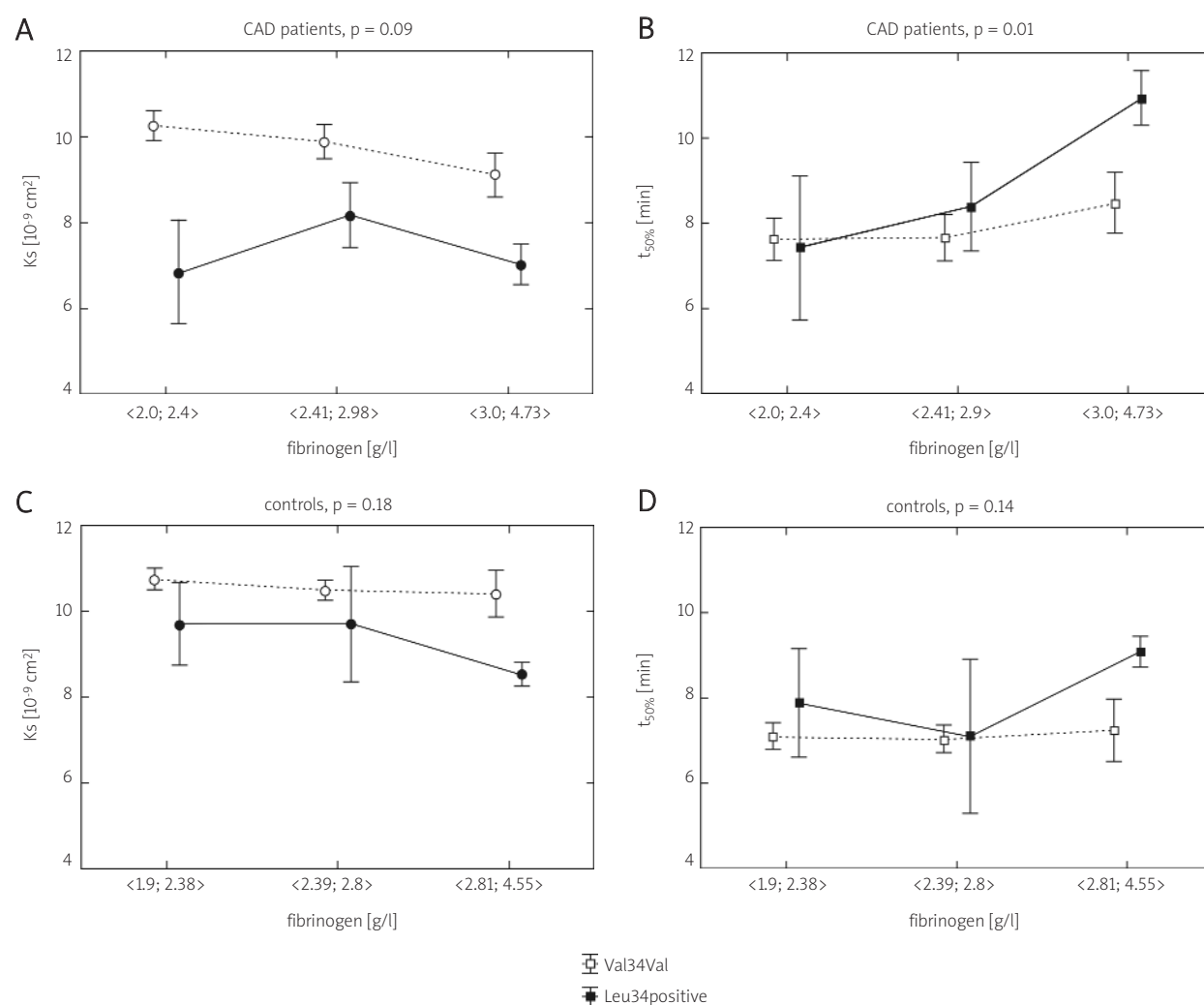
Fibrinogen

In the CAD group Leu34 carriers showed lower clot permeability in each tercile of fibrinogen concentration than those homozygous for Val34 allele (6.83 ± 1.89 vs. $10.25 \pm 1.01 \times 10^{-9}$ cm² in the lower tercile, 8.16 ± 1.18 vs. $9.88 \pm 1.00 \times 10^{-9}$ cm² in the median and 7.02 ± 0.99 vs. $9.11 \pm 1.24 \times 10^{-9}$ cm² in the upper tercile, $p < 0.001$). Factorial ANOVA analysis showed that the fibrinogen

Table II. Values of selected laboratory variables in controls and CAD patients in relation to the Val34Leu FXIII polymorphism

Variable	Controls (n = 96)		CAD patients (n = 113)	
	Homozygous for allele Val34 (n = 68)	Leu34 carriers (n = 28)	Homozygous for allele Val34 (n = 82)	Leu34 carriers (n = 31)
K_s [10^9 cm^2]	10.58 ± 0.80	$8.65 \pm 0.15^*$	9.86 ± 1.70	$7.29 \pm 1.90^*$
$t_{50\%}$	7.10 ± 0.60	$8.93 \pm 0.26^*$	7.86 ± 1.50	$9.96 \pm 3.35^*$
tHcy [$\mu\text{mol/l}$]	10.81 ± 1.30	$14.15 \pm 6.90^*$	11.69 ± 2.80	$17.93 \pm 7.75^*$
Lp(a) [mg/dl]	9.93 ± 4.70	$17.65 \pm 7.65^*$	15.65 ± 11.0	$27.34 \pm 13.30^*$
CRP [mg/l]	1.43 ± 0.66	$2.17 \pm 1.04^*$	1.55 ± 0.81	$2.67 \pm 1.03^*$
Fbg [g/l]	2.45 ± 0.27	$3.25 \pm 0.63^*$	2.67 ± 0.63	$3.46 \pm 1.44^*$

Abbreviations: Fbg – fibrinogen, Lp(a) – lipoprotein (a), CRP – C-reactive protein, Hcy – total homocysteine

* $p < 0.0001$ **Figure 1.** Factorial ANOVA analysis of K_s and $t_{50\%}$ parameters with regard to the factor XIII Val34Leu polymorphism and fibrinogen concentrations. Panels A and B show data for CAD patients. Panels C and D show data for controls

concentration does not modulate the Leu34 allele influence on the clot permeability ($p = 0.09$) (Figure 1 A). In the Leu34 carriers with CAD, $t_{50\%}$ was significantly longer in the upper tercile of fibrinogen concentrations alone (10.92 ± 1.36 vs. 8.46 ± 1.98 min; $p < 0.0001$), while in the lower and the medial terciles $t_{50\%}$ did not differ in Leu34 carriers and those homozygous for Val34 allele (7.43 ± 0.58 vs. 7.62 ± 1.30 min and 8.38 ± 1.2 vs. 7.64 ± 1.49 min, respectively NS). Factorial ANOVA analysis showed that fibrinogen modulates the Leu34 allele influence on lysis parameters in CAD patients ($p = 0.01$) (Figure 1 B).

In the control group the a fibrinogen concentration affects in a similar way the permeability and the lysis time in homozygotes Val34Val and Leu34 carriers. Clot permeability was lower in Leu34 carriers in every fibrinogen tercile (9.70 ± 0.14 vs. $10.75 \pm 0.50 \times 10^{-9}$ cm², 9.70 ± 0.0 vs. $10.49 \pm 0.54 \times 10^{-9}$ cm² and 8.53 ± 0.99 vs. $10.42 \pm 0.39 \times 10^{-9}$ cm², $p < 0.0001$). Lysis time was significantly longer only in the upper tercile of fibrinogen concentrations (9.08 ± 1.52 vs. 7.25 ± 0.72 min, $p < 0.0001$), while in the lower and the median terciles $t_{50\%}$ did not differ between FXIII Leu34 carriers and Val34Val homozygotes (7.90 ± 0.28 vs. 7.10 ± 0.56 min and 7.10 ± 0.0 vs. 7.05 ± 0.46 min, respectively NS) (Figure 1 C, D).

Homocysteine

In CAD patients, total Hcy levels alters clot permeability resistance to lysis regardless of the FXIII Val34Leu polymorphism. Factorial ANOVA showed no associations between the presence of Leu34 allele and the Hcy-related decrease in K_s and an increase in $t_{50\%}$. The clot permeability decreased significantly in each tercile of Hcy concentrations in subjects with the Val34Val genotype (10.36 ± 0.71 , 10.12 ± 0.88 , $8.26 \pm 0.99 \times 10^{-9}$ cm²; $p < 0.0001$) and the Leu34 carriers (9.70 ± 0 , 8.41 ± 0.87 , $6.80 \pm 0.91 \times 10^{-9}$ cm²; $p < 0.0001$). Similarly, lysis time increased in the CAD group both in patients homozygous for Val34 allele in each tercile (6.95 ± 0.57 , 7.64 ± 0.92 , 10.23 ± 1.68 min; $p < 0.0001$). In the Leu34 carriers with CAD lysis time increased in the same way (7.10 ± 1.0 , 8.60 ± 1.31 , 10.55 ± 1.78 min; respectively $p < 0.0001$).

In the Leu34 positive controls, K_s decreased gradually with an increase in total Hcy concentrations, reaching the lowest level in the upper tercile of Hcy concentrations (9.67 ± 0.09 , 8.74 ± 0.94 , and $8.38 \pm 1.02 \times 10^{-9}$ cm², $p = 0.0001$, respectively). The Val34Val patients did not show differences in clot permeability in terciles of Hcy concentrations (10.72 ± 0.40 , 10.10 ± 0.47 , and $8.26 \pm 0.69 \times 10^{-9}$ cm², NS). Factorial ANOVA analysis showed that Hcy levels modulate clot permeability only in the presence of Leu34 allele in controls ($p < 0.01$). Similarly, lysis time was associated with total Hcy levels and the Leu34 allele in the control group. A significant prolongation of $t_{50\%}$ was observed in relation to Hcy concentrations, with

the longest lysis time in the upper tercile (7.30 ± 0.54 , 8.47 ± 0.84 , 9.5 ± 1.57 min, respectively $p < 0.0001$). Factorial ANOVA analysis revealed that elevated total Hcy levels are linked to prolonged $t_{50\%}$ in the presence of the Leu34 allele in controls ($p = 0.003$).

C-reactive protein

In the CAD group Leu34 carriers showed lower clot permeability depending on CRP concentrations and no Leu34 carrier was included in the lower CRP tercile (8.60 ± 0.68 and $6.99 \pm 1.09 \times 10^{-9}$ cm², $p < 0.0001$). Patients homozygous for Val34 allele had gradually reduced K_s values in each tercile of CRP concentrations (10.58 ± 0.65 , 9.54 ± 0.73 and $8.53 \pm 1.56 \times 10^{-9}$ cm², $p < 0.001$). Lysis time was prolonged in both the Leu34 carriers and non-carriers with CAD in consecutive terciles of CRP concentrations (8.60 ± 1.54 and 10.27 ± 1.87 min vs. 7.03 ± 0.68 , 8.29 ± 1.43 and 9.02 ± 2.43 min; $p < 0.0001$). Factorial ANOVA analysis did not show any effect of Leu34 allele on fibrin permeability or lysis time in relation to CRP ($p = 0.4$) in patients with advanced CAD. In controls the influence of the FXIII Val34Leu polymorphism on both fibrin parameters was unaffected by CRP levels (data not shown).

Discussion

Our study shows that in patients with advanced CAD scheduled for CABG, the FXIII Leu34 allele was associated with decreased fibrin clot permeability and efficiency of clot lysis compared to the FXIII Val34Val genotype. Compared to apparently healthy control subjects, the impact of the FXIII Leu34 allele in severe CAD was similar in terms of permeability and almost identical while analyzing clot lysis time. The Leeds Family Study showed that genetic factors contribute modestly to variance in fibrin clot measures, including lysis time (range, 10-40%), while the contribution of environmental factors, being poorly characterised, is much larger [23]. We confirmed this observation. However in patients with severe CAD the effect of FXIII Leu34 allele is detectable also in the presence of elevated Hcy, Lp(a) and CRP levels [17-20] and in the presence of clinical confounders, i.e. previous MI or diabetes [5]. Interestingly, only diabetes tended to affect both fibrin variables in the clinical setting of severe CAD, which supports the view that the combined impact of cardiovascular risk factors and/or atherosclerotic burden produce a stronger effect on coagulation and the impact of individual risk factors could be hardly detectable. Well controlled chronic disorders (e.g. diabetes or hypertension) prior to surgery might also attenuate their impact on fibrin clot properties. Moreover, although aspirin at therapeutic doses has been reported to increase clot permeability and enhance fibrinolysis in healthy individuals [22, 24], FXIII Val34Leu polymorphism associated fibrin clot characteristics are present also in patients receiving aspirin.

Moreover, statins known to favorably alter fibrin clot properties [19, 25], did not abolish the effect of this polymorphism on fibrin features in CAD.

Of note, our study confirmed that advanced CAD is associated with markedly altered fibrin clot features. Since the first report on unfavorably altered clot features demonstrated in patients with advanced CAD in 1992 [26], it has been shown that about 50% of CAD patients have clot permeability below the 10th percentile of the control group [27] and fibrin clots with tightly packed fibers and small pores are associated with the number and severity of coronary artery stenoses documented by angiography [26]. Changes in clot characteristics reported in the current study are however significantly milder than those observed in patients evaluated within the first hours of myocardial ischemia probably largely due to lack of the effect ascribed to acute phase reactants [21].

Clot structure, which can be studied in solutions of purified fibrinogen and plasma, is characterised by several indirect measures such as (1) clot permeability, or Darcy constant K_s (an indicator of the pore size), calculated based on the volume of buffer flowing through the fibrin gel in a given time period; (2) fiber mass-length ratio, based on the permeability and the fibrinogen molar concentration; (3) lag phase by turbidimetry that reflects the time to the start of lateral fibril aggregation; (4) maximum absorbancy of the growing clot that reflects average fibrin fiber size and the number of protofibrils per fiber [28, 29]. Using scanning electron microscopy, fiber diameter can also be measured. However, this technique requires fixing a clot mostly by permeating it with glutaraldehyde solution with the subsequent dehydration and they are performed on a limited number of samples [5]. This analysis includes only fibers with clearly defined margins. Thromboelastography is another technique used to assess a global function of hemostasis and also to describe elastic properties of the clot during fibrin polymerisation [30]. We used a pressure-driven permeation method to measure permeability, which has been successfully applied in several papers published by our group [17-21, 31]. Resistance to plasmin-mediated proteolysis can be assessed by measurement of the half lysis time, defined as the time after which the absorbency of the clot decreases by 50% of the peak value [21, 22], or via determination of D-dimer concentrations in the buffer percolating through the fibrin gel [19, 32]. Our approach with determination of $t_{50\%}$ has also been used for more than 10 years and has low variability of results [17, 19, 20, 31].

It has been reported that the FXIII Val34Leu polymorphism is associated with fibrin structure and function in relation to fibrinogen concentrations. In contrast to the study by Lim et al. [6], we have not observed such relationships, which indicates that advanced atherosclerotic vascular disease, associated with the presence of several risk factors with the potential to

unfavorably alter blood coagulation, abolishes the distinct effect of fibrinogen concentrations on clot permeability. Of note, we extended findings reported by Lim et al. [6] beyond permeability measurements by showing that patients with advanced CAD did not show any relationship of clot lysis time and fibrinogen concentrations either.

The prevalence of Leu34 allele in Polish population was previously reported by Słowik et al. [11]; they found that the Leu34 allele frequency in stroke patients was 25.8%. We have obtained similar results with the Leu34 allele prevalence of 25% in CAD patients and 18% in controls ($p = 0.23$).

Several limitations of the current study should be acknowledged. First, our study was performed on a small sample size with regard to genetic association analysis. However, the data are consistent with the available data obtained in purified systems of using different methodological approaches. Characteristics of the patients are typical of subjects scheduled for CABG in the real life, therefore our findings might be extrapolated to larger patient populations. Second, we performed cross-sectional analysis at one time point. Third, unexpectedly Leu34 carriers displayed higher levels of confounders known to affect fibrin clot properties, e.g. Hcy compared to the non-carriers, not to mention age. Finally, we did not follow patients to investigate potential clinical relevance of the current results. A larger study is needed to address this issue.

In conclusion, our findings suggest that the FXIII Val34Leu polymorphism affects fibrin clot network structure and efficiency of fibrinolysis in patients with severe CAD subjected to the activity of several potent fibrin clot modulators.

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Polimorfizm czynnika XIII Val34Leu jako cecha wpływająca na przepuszczalność skrzepu fibrynowego i jego oporność na lizę u osób z zaawansowaną chorobą wieńcową

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Streszczenie

Wstęp: Częsta transycja G na T w kodonie 34 z następczą zmianą waliny przez leucynę w podjednostce A czynnika XIII (FXIII) wpływa na proces powstawania fibryny i jej stabilizację *in vitro*. Dane o wpływie allele Leu34 FXIII na zakrzepowo-zatorowe incydenty sercowo-naczyniowe *in vivo* nie są jednoznaczne.

Cel: Zbadanie, czy polimorfizm FXIII Val34Leu jest wystarczająco silny, aby zmienić właściwości skrzepu fibrynowego u pacjentów z zaawansowaną chorobą wieńcową (CAD).

Metody: Badanie objęło 113 chorych w wieku $62,8 \pm 6,1$ roku zakwalifikowanych do elekttywnej izolowanej operacji pomostowania aortalno-wieńcowego (CABG). Pacjentów porównano z 98 zdrowymi osobami dobranymi pod względem wieku. Przepuszczalność skrzepu fibrynowego i czas jego lizy ($t_{50\%}$) mierzono *ex vivo* w osoczu cytrynianowym.

Wyniki: Pacjenci zakwalifikowani do CABG cechowali się mniejszą przepuszczalnością skrzepu ($9,14 \pm 1,64$ vs $10,02 \pm 1,12 \times 10^{-9} \text{ cm}^2$; $p = 0,0002$) i dłuższym $t_{50\%}$ ($8,45 \pm 1,94$ vs $7,63 \pm 1,24$ min; $p < 0,0001$) niż osoby z grupy kontrolnej. Nosiciele allele Leu34 FXIII, tj. 9 (8%) homozygot Leu34Leu i 23 (20%) heterozygoty Val34Leu, cechowali się mniejszą przepuszczalnością skrzepów o 23% w grupie CAD ($p < 0,0001$) w porównaniu z 81 (72%) osobami homozygotycznymi pod względem allele Val34. Podobne różnice między grupami obserwowano, analizując $t_{50\%}$, który był dłuższy u nosicieli allele Leu34 ($p < 0,0001$). Częstość występowania allele Leu34 była podobna w grupie kontrolnej, tak jak jego oddziaływanie na właściwości fibryny. Wpływ allele FXIII Leu34 na przepuszczalność skrzepu nie zależał od stężenia homocysteiny, białka C-reaktywnego i fibrynogenu u chorych z CAD oraz osób z grupy kontrolnej.

Wnioski: Podobnie jak u zdrowych osób, u chorych zakwalifikowanych do CABG obecność allele FXIII Leu34 wiąże się ze zmniejszoną przepuszczalnością skrzepu fibrynowego i sprawnością lizy.

Słowa kluczowe: czynnik XIII, fibrynoliza, choroba wieńcowa, polimorfizm

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