THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D.)

FACTOR XIII AND ITS VAL34LEU POLYMORPHISM IN ATHEROTHROMBOTIC DISEASES

By Amir-Houshang Shemirani, MD

Supervisor:

Prof. László Muszbek, MD, Ph.D., D.Sc., M.H.A.Sc.

UNIVERSITY OF DEBRECEN

Kálmán Laki Doctoral School
of
Thrombosis, Hemostatis, and Vascular Diseases

Debrecen, 2011

Table of Contents

ABBREVIATIONS

FXIII: Blood coagulation factor XIII

FXIII-A: Blood coagulation factor XIII A subunit

FXIII-B: Blood coagulation factor XIII B subunit

cFXIII: Cellular FXIII

FXIIIa: active FXIII

A_2PI: α_2-plasmin inhibitor

FRET: Fluorescence resonance energy transfer

CAD: Coronary artery disease

tPA: Tissue plasminogen activator

BACKGROUND

The plasmatic form of blood coagulation factor XIII (FXIII) is a zymogen (protransglutaminase) of tetrameric structure (A_2B_2) (1-3). It contains two potentially active A subunits (FXIII-A) and two inhibitory/carrier B subunits (FXIII-B). Cellular FXIII (cFXIII) is a homodimer of FXIII-A, and exists in vast amounts in platelets and megakaryocytes (4, 5), Cellular FXIII is also present in monocytes, in their bone marrow precursor cells and in monocyte-derived macrophages including tissue macrophages (histiocytes) (6, 7). FXIII-A is synthesized by cells of bone marrow origin, while FXIII-B is synthesized by hepatocytes and the two subunits form a tetrameric complex in the circulation. In the plasma FXIII-A is present only in complex, while FXIII-B and is in excess, approximately 50% of its total amount exists in free form. The reference interval for plasma FXIII-A_2B_2 concentration is 14-28 mg/L (8). FXIII-A consists of an activation peptide (1-37 amino acids), one β-sandwich (38-184 amino acids), one catalytic ("core" domain, 185-515 amino acids) and two β-barrels (516-628 and 629-730 amino acids) (9). FXIII-B is a mosaic protein, it consists of 10 "sushi" domains, each held together by a pair of disulphide bridges.

Plasma FXIII is transformed into an active transglutaminase (FXIIIa) by the proteolytic action of thrombin in the presence of Ca^{2+}. Thrombin removes the activation peptide from FXIII-A, then in the presence of Ca^{2+} FXIII-B dissociates and FXIII-A assumes an enzymatically active configuration (FXIII-A') (Figure 1) (1, 6). The presence of fibrin greatly accelerates the activation process.

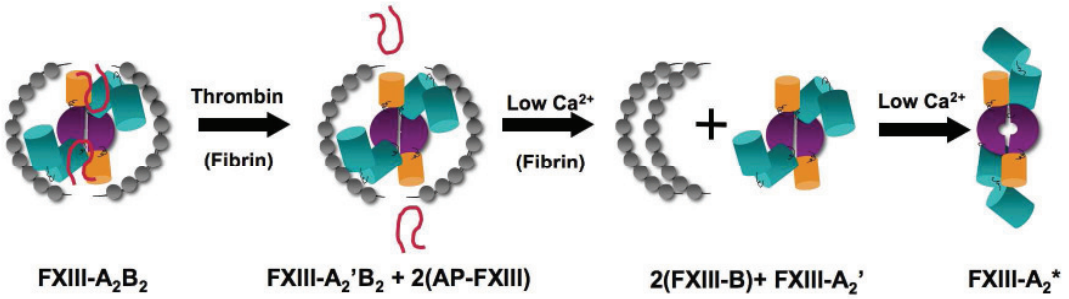

Figure 1. Activation of plasma factor XIII. (The Figure was constructed by Dr. Zsuzsa Bagoly Clinical Research Center, University of Debrecen, Medical and Health Science Center, and was taken from the publication of Muszbek et al. (10).

The zymogen, factor XIII, is a tetramer (A_2B_2) composed of two identical A subunits and 2 identical B subunits. Thrombin cleaves a Mr~4000 Da peptide from the NH_2-terminus of FXIII-A. Full expression of activity is achieved following Ca^{2+}-induced dissociation of FXIII-B and the conformational change of FXIII-A_2. Green and orange cylinders represent β-barrel and β-sandwich domains of FXIII-A, respectively. The central core domains in FXIII-A are depicted as horseshoes in magenta. The activation peptides are shown as red loops. The elongated bended structure consisting of ten pearls surrounding FXIII-A_2 corresponds to FXIII-B.

Active transglutaminases catalyze an acyl-transfer reaction (1). In the first step a peptide-bound glutamine residue forms a thioacyl intermediate with the active site Cys314 and ammonia is released. In the absence of an amine substrate hydrolysis of the thioacyl intermediate occurs and the peptide bound glutamine becomes deamidated. If a substrate primary amine is present, the acyl group is transferred to the acyl acceptor primary amine and the amine becomes linked to the glutamyl residue through an isopeptide bound. If the substrate amine is provided by the ε-

amino group of a peptide bound lysine residue an ε(γ-glutamyl)lysyl is formed and peptide chains become covalently cross-linked (11). The activation of FXIII takes place on the surface of newly formed fibrin; the truncated active dimer remains associated with fibrin and no thrombin-cleaved FXIII could be detected in the serum (12).

Factor XIII is essential for maintaining hemostasis, its deficiency causes severe bleeding diathesis, in non-substituted patients frequently with fatal brain hemorrhage (13). The primary physiological substrates of FXIIIa are fibrin and α_2-plasmin inhibitor (α_2PI) (14). Accordingly, the main biochemical task of FXIII in the normal hemostasis is to cross-link fibrin chains and to covalently attach proteins important in the regulation of fibrinolysis to the newly formed fibrin network. Cross-linking by FXIIIa improves the mechanical strength, rigidity and elasticity of the clot and increases its resistance to fibrinolysis (15, 16). FXIIIa cross-links fibrin γ- and α-chains and the major cross-linked products are γ-chain dimers and high molecular weight α-chain polymers (17). γ-chain dimer formation is an extremely quick process, requires only minute amount of FXIIIa and immediately follows the removal of fibrinopeptide A from fibrin (18). The multiple cross-linking of α-chains among several acyl donor and acyl acceptor sites proceeds more slowly than γ-chain dimer formation. Besides γ-chain dimers and α-chain polymers γ-α chain heterodimers and γ-chain trimers/tetramers are also formed (19-21). α_2PI is an excellent acyl donor substrate for FXIIIa and it can be cross-linked to the α-chain of fibrin and fibrinogen rapidly (14). Its covalent attachment to fibrin plays a primary role in preventing the prompt elimination of newly formed fibrin by the powerful fibrinolytic system. FXIIIa has a number of other potential substrate proteins in plasma, (22-28) and there are known FXIIIa substrates among cellular proteins, as well (29-35). However, the physiological importance of these cross-linking reactions still needs to be elaborated.

The physiological role of FXIII is unusually diverse for a clotting factor. In addition to hemostasis, FXIII has important functions on at least three major closely related, yet distinct areas. It promotes angiogenesis, it is important for wound healing and plays an essential role in maintaining pregnancy (36-38).

The gene coding for human FXIII-A (F13A1) is located at chromosome 6p24-25, it contains 15 exons and 14 introns and spans over 160 kb (39, 40). Exon I consists of the 5' noncoding region, exon II encodes AP-FXIII. The β-sandwich domain, the catalytic core domain and the two β-barrel domains are encoded by exons II-IV, exons IV-XII, exons XII-XIII and exons XIII-XV, respectively. It is transcribed into a 3.9-kb mRNA, with an 84-base pair 5' untranslated region, a 2.2-kb open reading frame and a 1.6-kb 3' untranslated region.

The gene of FXIII-B is located at 1q31-32. The gene of FXIII-B subunit (F13B) is located at position 1q31-32.1, it is composed of 12 exons producing a 2.2 kb mRNA (41). Exon I encodes a 20 amino acid leader sequence, exon II-XI encode for the sushi domains, each sushi domain is encoded by a single exon. The last exon codes for a COOH-terminal region of FXIII-B, for the 3'-untranslated region and for the polyA tail. The expression of F13B is directly regulated by transcription factors HNF1α and HNF4α (42, 43).

The following common polymorphisms with amino acid exchange have been described in FXIII-A: Val34Leu, Tyr204Phe, Leu564Pro, Val650Ile and Glu651Gln, among which the Val34Leu polymorphism, first described by Mikkola et al. (44) has stirred considerable interest, because of its suspected thrombo-protective effect. The frequency of Leu34 allele among Caucasians is around 25%, in Africans its frequency is significantly lower and in Asians it is extremely rare (45). The concentration of plasma FXIII in subgroups with different Val34Leu genotypes does not differ significantly (46) indicating that the rate of secretion of FXIII-A

variants and their lifespan in the plasma are similar. Considering that the location of this polymorphism is just 3 amino acids up-stream from the thrombin cleavage site, one would expect its influence on thrombin-induced FXIII activation. Indeed, it was demonstrated with both cFXIII (47) and pFXIII (46, 48) that the thrombin induced release of AP-FXIII from the Leu34 FXIII-A variant, as well as the consequent activation of FXIII proceed at a 2.5-folds higher rate than in the case of Val34 variant. This finding was also supported by NMR studies testing the binding of the synthetic AP-FXIII segment (28-41) to thrombin (49-51). Testing six single-nucleotide polymorphisms in the FXIII-A gene indicated that indeed Val34Leu is the main functional polymorphism influencing FXIII activation (52). Faster activation of FXIII results in accelerated fibrin cross-linking and in a higher rate of α_2PI incorporation into fibrin (46-48, 53). These experiments were carried out with purified proteins and it was not known if such a relationship between FXIII-A Val34Leu polymorphism and FXIII activation also exists in the more complex environment of human plasma.

Although for a while the effect of the Val34Leu polymorphism on the specific activity of FXIIIa was controversial, it is now clear that the specific activity of fully activated pFXIII (46, 48), cFXIII (47) and recombinant FXIII-A_2 (54) of different FXIII-A Val34Leu genotypes are identical. In a few studies, measurement of FXIII activity was performed with FXIII only partially activated by thrombin. In these cases the measured activity reflected the rate of FXIII activation rather than the catalytic concentration of FXIII and higher values were obtained for the Leu34 variant than for the Val34 variant. In conclusion, neither plasma FXIII concentration nor the transglutaminase activity of FXIIIa is influenced by FXIII-A Val34Leu genotype. The rate of activation is, however significantly faster in the case of the Leu34 variant.

Further interesting findings were, that the structure of fibrin clots is influenced by FXIII-A Val34Leu polymorphism,(48) and this effect is modulated by the concentration of fibrinogen (55). At high fibrinogen concentrations, plasma samples from homozygotes for the Leu34 allele form clots having looser structure, thicker fibers and increased permeability, while at low fibrinogen concentrations fibrin meshwork had thinner, more tightly packed fibers and lower permeability. Practically no fibrinogen concentration dependent changes were observed in the plasma samples of wild type individuals.

The biochemistry of other common FXIII-A polymorphisms has not been investigated in such details as that of Val34Leu polymorphism. The Phe204 allele of FXIII-A Tyr204Phe polymorphism was reported to be associated with decreased pFXIII level and activity, whereas the Leu564 allele of the Pro564Leu variant resulted in lower FXIII plasma level with increased FXIII activity (56, 57). However, these results still need to be confirmed.

The polymorphic nature of FXIII-B has been revealed by isoelectric focusing experiments a long time ago (41, 58). Based on these experiments FXIII-B was classified into three major population-associated genotypes (European, African, Asian), plus several rare alleles. Two major FXIII-B polymorphisms have been described, His95Arg substitution in the second sushi domain (59), and C-to-G change at position 29756 in intron K29756 resulting in a novel splice acceptor site (60, 61). The frequency of FXIII-B Arg95 carriers among healthy Caucasians is about 15%; it is more frequent among Africans and is missing from the Asian population (59, 61). The polymorphism did not influence FXIII-A, FXIII-B and pFXIII antigen levels (59). In the plasma of individuals carrying the Arg95 allele, FXIII-A_2B_2 antigen levels were significantly decreased following thrombin activation that might be due to increased dissociation of the subunits. However, these results could not be confirmed by steady-state

binding experiments using purified FXIII subunits (59) and therefore, further investigations are needed to draw a final conclusion. The other polymorphism results in allele-specific splicing products and a protein 15 amino acids longer at the C-terminus than its wild type counterpart is synthesized (61). The polymorphism characteristically occurs in Asian, to a lesser extent in Caucasian populations, and it does not seem to be present among Africans. Although such a profound structural change would be expected to alter some of the biochemical features of the molecule, this possibility has not been explored. The same stands for a rare allele resulting in Glu368 to Val substitution.

Since the discovery of the first FXIII deficient patient (62) a number of studies explored the genetic background, the clinical consequences of FXIII deficiency and studies on FXIII-A knock-out mice further provided further details on the mechanism of the disease (see reviewed by Karimi et al. (13)) Interestingly, the relationship between FXIII and the risk of thrombotic diseases, in spite of gradually increasing number of studies in the last one and half decade, is less clear. The association of FXIII with the risk of thrombosis includes both major group of thrombotic diseases, venous thromboembolism (VTE) and atherothrombotic diseases. Our interest concerned the latter group and we reviewed the data published in the literature in an article of Seminars in Thrombosis and Hemostasis (63). The effect of elevated FXIII level was investigated only in a few studies; the largest among them involving 955 patients (377 females and 578 males) was published by our laboratory (64). In this study patients were sub-grouped according to the presence or absence of CS and according to the presence or absence of positive history of MI. In females, but not in males, adjusted FXIII levels were moderately, but significantly elevated in patients with CS and the history of MI. CS itself was without effect on FXIII levels. FXIII activity and antigen levels in the upper tertile represented a 2.5-3.0-fold risk

of CS with MI in females, but not in males. Elevated FXIII level did not increase the risk of CS, while significantly increased the risk of MI in females suffering from CS. The latter finding suggests that the elevation of plasma FXIII level increases the risk of MI by mechanisms other than affecting the development of atherosclerotic plaques.

The first pioneering case-control study on the association of FXIII-A Val34Leu polymorphism with the risk of CAD and MI was published in 1998 by Kohler et al. (65). They demonstrated a protective effect of FXIII-A Val34Leu polymorphism against MI in a Caucasian patient population admitted for routine coronary angiography. Since then, twenty one studies have been reported; eight confirmed the original report, while in 13 studies Leu34 carriership did not confer significant protection or risk to the studied populations; for details consult reference (63). The number of contradictory reports indicated the necessity of a meta-analysis of the reported data, which was performed in 2007 in our laboratory. The meta-analysis of 16 studies involved 5346 cases and 7053 controls (66). The combined odds ratios for CAD were 0.82 (95%CI: 0.73, 0.94) for Leu34 heterozygotes, and 0.81 (95%CI: 0.70, 0.92) for the heterozygotes and homozygotes combined. The results were essentially the same when only MI was considered as outcome. A protective effect of Leu/Leu genotype was also indicated (OR: 0.89, 95%CI: 0.69, 1.13) but, likely due to the low frequency of this genotype, the results were not statistically significant. The meta-analysis suggested that there is an association between the FXIII-A Leu34 allele and a relatively modest, but significant protective effect against CAD.

The wide range of data published in the literature and the results of meta-analysis suggest that gene-gene and gene-environmental interactions might significantly influence the effect of FXIII-A Val34Leu polymorphism. The results of several recent studies support such a hypothesis. In young women with obesity the presence of Leu34 allele provided significant

protection against MI (OR: 0.33, 95%CI: 0.13, 0.83) while in non-obese subject it did not (OR: 1.68, 95%CI: 0.8, 3.51) (67). The interaction among PAI-1, insulin resistance and FXIII-A Val34Leu polymorphism represents another example. In subjects possessing the Leu allele insulin resistance and elevated PAI-1 level, related to 4G/5G polymorphism in the PAI-1 gene, led to the loss of cardioprotection (68, 69). As it has been discussed earlier FXIII-A Val34Leu polymorphism alters the structure of fibrin network and influences its fibrinolytic degradation in a fibrinogen concentration-dependent manner (48, 55, 70). Thus, the interaction between fibrinogen level and FXIII-A Val34Leu polymorphism is expected to be important in the determination of the risk of CAD. Two studies have addressed this question. It was shown by Boekholdt et al. that in a population where the overall effect of Val34Leu on the risk of CAD was negligible, at fibrinogen concentration in the lowest tertile the Leu/Leu genotype represented a risk of CAD (OR: 2.88, 95%CI: 1.24, 6.74), while at fibrinogen concentrations in the highest tertile an opposite tendency (OR: 0.47, 95%CI: 0.18, 1.17) was observed (71). The presence of FXIII–A Leu34 in homozygous or heterozygous form did not change the risk of CS or MI in the general Hungarian population (72). However, for patients with fibrinogen level in the upper quartile, a statistically significant protection against MI (OR: 0.41, 95%CI: 0.18, 0.93) was conferred by carrying the Leu34 allele. The limited amount of data on the relationship of CAD/MI and FXIII-A polymorphisms other than Val34Leu and FXIII-B polymorphism does not allow a clear evaluation (63).

The association between FXIII levels and FXIII polymorphism with the risk of other atherothrombotic diseases, PAD and AIS, is much less investigated than the association with the risk of CAD. As part of our studies deal with this topic the data available in the literature will be reviewed and compared with out data in the Discussion.

The aims of my PhD studies were the followings:

1/ To develop a method for the detection of FIII-A Val34Leu polymorphism, which is applicable for the quick, reliable analysis of a large number of DNA samples, including samples isolated from postmortem material.

2/ To establish if the effect of FXIII-A Val34Leu polymorphism on FXIII activation, which has been investigated on purified proteins, also prevails in the more complex environment of human plasma.

3/ To reveal the association of FXIII levels and FXIII-A Val34Leu polymorphism with the risk of PAD.

4/ To reveal the association between FXIII-A Val34Leu polymorphism and the risk of AIS with non-fatal or fatal outcome.

MATERIALS, METHODS AND PATIENTS

Development of a melting point analysis method, using fluorescence resonance energy transfer detection (FRET), for the identification of FXIII-A Val34Leu polymorphism

DNA was isolated from citrated whole blood of 113 unrelated subjects [68 wild-type (V/V), 36 heterozygote (V/L), 9 homozygote (L/L)] using MagNA Pure LC Instrument (Roche Diagnostics). Primers and probes were synthesized by TIB MOLBIOL.

The following primers and probes were used for genotyping FXIII-A Val34Leu polymorphism:

Primers/Probes	Sequence
Forward primer	5'-GAC CTT GTA AAG TCA AAA ATG TC-3'
Reverse primer	5'-AA-GGG-GGG-TAT-GCT-CAT-3'
Sensor	5'-G-CAC-A*AC-GCC-CTG-AAG-C-3'FL[a,b]
Anchor	5'-LC Red640-C-CAC-TGT-GGG-CAG-GTC-ATC-TTC-C-3'-P[a]

[a] FL, fluorescein; P, phosphorylated

[b] the site of the mutation is underlined; in the sensor a mismatch (C→A) was introduced at the position labeled with asterisks.

The detection probe (sensor) labeled with fluorescein at the 3' end covers the polymorphic site. A C→A mismatch was introduced in the sensor to decrease the melting temperature for both alleles. The adjacent anchor probe was 5' labeled with the LC-Red640 dye and the 3' end was phosphorylated to prevent probe elongation by Taq polymerase. The gap between the two probes is a single nucleotide. If the probes lie adjacent to each other on a DNA strand, fluorescence resonance energy transfer occurs and the fluorescence of LC Red640 is detected by the fluorimeter component of LightCycler.

PCR reactions were performed in the LightCycler glass capillaries in a final volume of 21 µl containing 150 ng of genomic DNA, 0.19 [µmol/l] of each primer, 0.57 [µmol/l] of anchor probe, 0.38 [µmol/l] of sensor probe, 2 U Taq DNA polymerase (Roche), 2 µl of 10 x concentrated PCR reaction buffer (Roche), 42.9 ml/l dimethyl sulfoxide (Sigma), 0.48 [mmol/l] of each dNTP (Roche), plus an extra 2.86 [mmol/l] of $MgCl_2$ (Roche). One sample without DNA, as a negative control, was included in all assay series. Cycling conditions were as follows: initial denaturation at 95°C for 30 s, 60 cycles of denaturation at 94°C for 0 s, annealing at 50°C

for 5 s and extension at 72°C for 10 s. Melting curve analysis consisted of one cycle at 94°C for 15 s and at 35°C for 15 s, followed by increasing the temperature to 70°C at a 0.1°C/s ramping rate. The fluorescent signal of LC Red640 was monitored continuously during the temperature ramp and the results were transformed to derivative melting curves.

Activation of FXIII in plasma and the recovery of fibrin

9 ml blood was collected in 1 ml 0.105 M trisodium citrate and platelet poor plasma was collected by centrifugation. Among the 23 selected healthy individuals (9 males, 14 females, age 22-45) 7 were wild type, 8 heterozygous and 8 homozygous for Val34Leu polymorphism. Val34Leu genotype was determined by the real-time polymerase chain reaction method described above. Highly purified human FXIII was prepared from the plasma of healthy volunteers with wild-type and homozygous Leu34 FXIII-A genotype according to Lorand et al (73). Human thrombin (1690 U/mg) was from Sigma (St.Louis, MO, USA). FXIII deficient plasma was purchased from Sigma and Trinity Biotech (Bray, Ireland).

100 μl of normal plasma of different FXIII-A Val34Leu genotypes were incubated with 0.1-0.3 U/ml thrombin and 18 mM $CaCl_2$ at 37°C. After various intervals the reaction was stopped by an equal volume of inhibitor cocktail containing 50 mM enthylenediaminetetraacetic acid, 20 mM benzamidine, 50 mM ε-aminocaproic acid, 2 mM iodoacetamide, 0.1 mM d-phenylalanyl-l-prolyl-l-arginine chloromethyl ketone, 100 mM NaCl and 50 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, pH 7.5 to block thrombin and FXIIIa, and to prevent fibrinolysis. The samples were centrifuged; the supernatants were removed and added to 9 volumes of sodium dodecyl sulfate-polyacrilamide gel electrophoresis (SDS-PAGE) sample

buffer. The remaining fibrin clot, if present, was washed with physiological NaCl solution. After dissolving the clot in 1 ml SDS-PAGE sample buffer its protein content was determined and $T_{1/2}$ for fibrin formation (the time between the addition of thrombin and the transformation of 50% of fibrinogen into fibrin polymer) was calculated. Similar experiments were also performed on two different batches of FXIII deficient plasma substituted with 20 μg/ml highly purified FXIII of Val34Val or Leu34Leu genotype. For the experiments demonstrated on the figures 0.17 U/ml thrombin was used. Varying thrombin concentrations in the range of 0.1-0.3 U/ml did not change the tendency of the results. At thrombin concentrations higher than 0.3 U/ml the clotting of plasma and the activation of FXIII was so quick that no meaningful evaluation of the time course of activation process could be carried out.

SDS-PAGE and Western blotting

Aliquots of denatured plasma/serum and fibrin samples were analyzed by SDS-PAGE. After SDS-PAGE part of the samples were electroblotted to Immobilon P membrane (Millipore, Bedford, MA, USA). FXIII-A and FXIII-A' were detected by rabbit anti-FXIII-A antibody (Calbiochem, San Diego, CA, UAS). The immuno-reaction was developed by Vectastain Elite ABC kit (Vector, Burlingame, CA, USA) and visualized by chemiluminescence (ECL Plus; Amersham, Little Chalfont, UK) according to the manufacturers' instructions. The relative amounts of FXIII-A and FXIII-A' were determined by quantitative densitometry using GS-800 Calibrated Densitometer (Bio-Rad, Hercules, CA, USA) and expressed as a percentage of the total FXIII present on the blot at full activation.

The time required for the activation of 50% of FXIII present in the plasma ($T_{1/2}$ FXIII-A') was calculated. The reproducibility of the assay was better than 5%. The amount of γ-chain dimers was determined by quantitative densitometry of the Coomassie-stained gels and the results were expressed as a percentage of fully cross-linked γ-chains.

Cases and controls

Study on peripheral artery disease (PAD)

302 consecutive PAD patients were recruited over a 2-year period from the 3rd Department of Medicine, University of Debrecen. Patients with history of myocardial infarction, stroke, symptoms of angina and renal insufficiency were excluded. The patients were exempt of acute inflammation during the last two months before blood drawing and did not have any chronic inflammatory state, other than PAD. Finally, 278 patients (173 males and 105 females) with ankle brachial pressure index (ABPI) ≤0.9 remained in the study group (173 with Fontaine classification II and 105 with Fontaine classification III). Patients were compared to sex-matched clinical controls (n=278) who also presented at the hospital, but no significant health problem, other than diabetes mellitus in some of the patients, was diagnosed and ABPI was in the range of 0.91-1.3. The mean age±SD was 59.0±9.5 in the control and 64.3±12.2 in the patient group. The occurrence of diabetes mellitus was 20% and 25% among controls and patients respectively. Thirty percent of controls and 36% of patients were smokers. The differences were not statistically significant. Two hundred and seventeen PAD patients received Aspirin and 33 patients received statins; since in the FXIII levels there was only a non-significant difference (<1%) between PAD patients on treatment and the rest of the patients, these groups were not

analyzed separately. Eighty-eight percent of women were menopausal; none of them on hormonal replacement therapy. Ethical approval for the studies was obtained from the Ethics Committee of the Medical and Health Science Center, University of Debrecen, Hungary. Individuals in the control groups and patients gave informed consent.

Study on patients surviving or not surviving atherothrombotic ischemic stroke (AIS)

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 these patients were enrolled at a military hospital and they had a relatively younger age and male dominance. The non-survivor group consisted of 316 patients with AIS, who died in the hospital within 4 weeks after admittance. In this group the diagnosis and the cause of death were confirmed by autopsy and formalin-fixed paraffin-embedded (FFPE) tissue specimens of the patients were stored in the Pathology Department of Diósgyőri Vasgyári Hospital, Miskolc, Hungary. Women involved in the study were not on hormonal replacement therapy.

The population control group that represented the general Hungarian population consisted of 1146 Hungarian individuals (72). The sampling frame for the reference group included all those registered with the participating practices in the Hungarian General Practitioners' Morbidity Sentinel Stations Program organized by the Department of Preventive Medicine, University of Debrecen, Faculty of Public Health. 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/or sex matched controls to the patient groups from this population control group. As the age and sex distribution in the groups of patients surviving and not surviving AIS differed significantly, separate matched control groups had to be selected for the two patient groups.

Ethical approval for the studies was obtained from the Ethics Committee of the Medical and Health Science Center, University of Debrecen, Hungary. Individuals in the control groups and patients gave informed consent.

Laboratory methods used in clinical studies

Plasma FXIII activity and antigen were measured by commercially available reagent kits (REA-chrom FXIII assay and R-ELISA FXIII, Reanal-ker, Budapest, Hungary) based on methods (8, 74) developed in our laboratory. In the measuring range, the CV for both assays was below 3%. Serum total cholesterol, LDL-cholesterol, HDL-cholesterol, triglyceride, apoAI, apo B, Lp(a) and high sensitivity C-reactive protein (hsCRP), plasma fibrinogen, homocysteine, folic acid and vitamin B_{12} were determined by routine laboratory methods and used for the determination of parameters independently associated with FXIII levels. Plasma fibrinogen level was measured by a modified Clauss method. Plasma homocysteine, folic acid and vitamin B_{12} were measured by immunoassays using AxSYM immunanalyser (Abbott Laboratories, Abbott Park, IL).

DNA from controls and patients was isolated from the buffy coat of citrated blood samples by QIAamp DNA Blood Mini Kit (Qiagen). DNA was also extracted from archival FFPE human brain tissues of patients died of fatal AIS. Five 10-µm sections were prepared from

FFPE blocks; de-paraffinization and DNA extraction were performed by EZ1 DNA Tissue Kit using EZ1 Advanced equipment (Qiagen) according to the manufacturer's protocol. Val34Leu polymorphism was determined by real time PCR using FRET detection and melting curve analysis on LightCycler equipment (Roche Diagnostics) using the method described above.

Statistical analysis

The Kolmogorov-Smirnov test was performed to examine the normality of the distribution of different parameters. Fibrinogen, triglyceride, HDL cholesterol, apo AI, hsCRP, homocysteine, folic acid and vitamin B_{12} were log-transformed to normalize the distribution. An independent Student's t test was used to assess differences in continuous data. A P value <0.05 was considered to indicate statistical significance. Correlation between ABPI and FXIII levels was determined by Pearson's method. A multiple linear regression analysis was performed for FXIII activity and antigen to determine the parameters independently associated with FXIII levels. The significance of differences in mean FXIII values between the clinical control and PAD patient were tested by analysis of variance (ANOVA). When one-way ANOVA indicated a significant difference, post-hoc pair-wise comparisons were made using the least significant difference test. The effect of elevated FXIII levels on the risk of PAD was also analyzed. The risk represented by FXIII activity, antigen levels being in the upper tertile, as compared to the rest of the patients, and the effect of FXIII-A genotype were expressed as the odds ratio (OR) and 95 percent confidence interval(CI), which were computed from the corresponding regression coefficient in the logistic regression model. χ^2 test was used for differences in category frequencies. Adjusted

ORs were obtained by the use of a model that included FXIII-A genotype and age. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS).

RESULTS

Evaluation of the newly developed melting point analysis method, using FRET detection, for the identification of FXIII-A Val34Leu polymorphism

In the Materials and Methods section we described a DNA-based method using hybridization probes, melting point analysis and FRET detection on LightCycler (Roche) for rapid identification of the FXIII Val34Leu polymorphism. Melting peak analysis easily discriminated the three different FXIII-A Val34Leu genotypes: melting temperature (Tm) for the wild-type (Val/Val) was at 56.4°C, while Tm for homozygote mutant (Leu/Leu) was at 47.5°C; heterozygotes demonstrated both peaks (Figure 2).

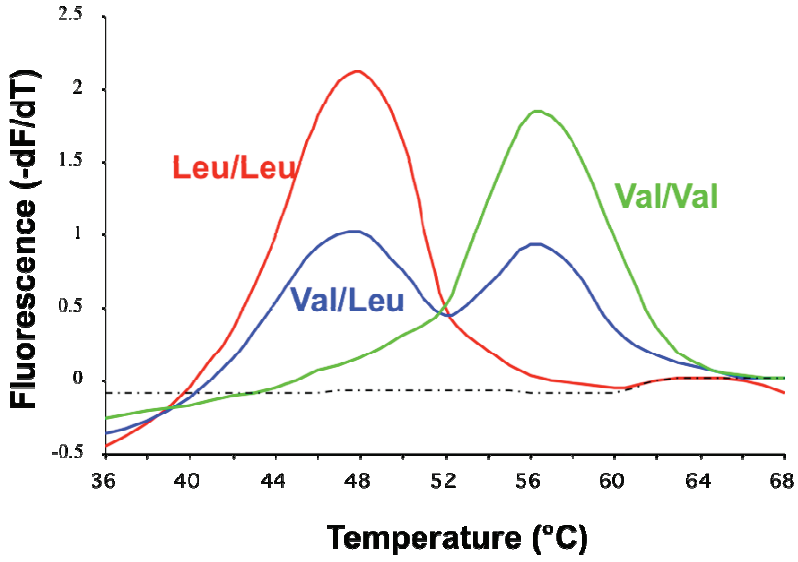

Figure 2. Representative derivative melting curves [-d(F2)/dT vs T] of the three FXIII-A Val34Leu genotypes. Green line, V/V; blue line, V/L, red line, L/L; thin dashed line, no template control.

113 individual DNA preparations from a sample pool were tested by the method described above and by PCR-RFLP according to Balogh et al. (46). The results were in 100% concordance. The frequency of the Leu34 allele was 24.2% (95% confidence interval: 16.3-32.1%) which well agrees with the allele frequency obtained by PCR-RFLP on a different group of the same ethnic Hungarian population (25.9%; 95% confidence interval: 22.3-29.5%) (46), and with the data reported for other Caucasian populations (45). 50 randomly selected DNA samples (26 wild type, 19 heterozygous and 5 homozygous for FXIII-A Val34Leu polymorphism) were also evaluated by fluorescent DNA sequencing using BigDye Terminator Reaction kit on an ABI 310 Genetic Analyzer (Applied Biosystems), again 100% concordance was observed. To test the reproducibility of the assay, DNA samples of all three genotypes were retested on at least 20 different occasions. All results were consistent.

The effect of FXIII-A Val34Leu polymorphism on the activation of FXIII in whole plasma

In collaboration with Dr. Bagoly (Zsuzsa Bagoly doctoral thesis, University of Debrecen 2008) we have shown that at a thrombin concentration that occur in physiological conditions the activation of FXIII takes place exclusively on the surface of fibrin and the truncated form (FXIII-A_2') never appears in the serum. Continuing this work, first I studied the quantitative correlation between fibrin clot formation and FXIII activation. Fig. 3A shows the Western blot for FXIII recovered in the fibrin clot. Once fibrin is formed (2 min after the addition of thrombin and Ca^{2+}), FXIII becomes attached to its surface and truncated by thrombin. The formation of fibrin is the initiator of FXIII activation and there is a highly significant correlation between the time

required for half maximal fibrin formation ($T_{1/2}$ for fibrin formation) and the time required for half maximal proteolytic activation ($T_{1/2}$) of FXIII (Figure 3B).

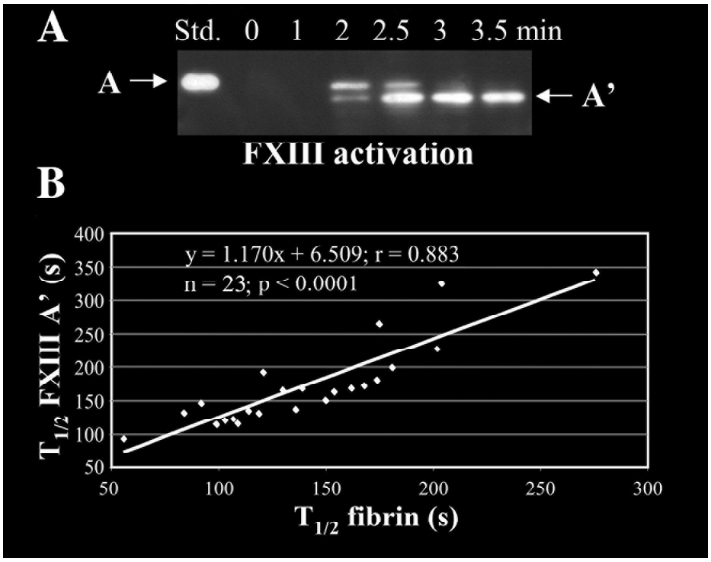

Figure 3. Fibrin formation and the proteolytic activation of FXIII by thrombin in whole plasma. (A) Intact FXIII-A subunit [A] and its truncated form [A'] on the Western blot of a representative fibrin sample obtained at different times after the addition of thrombin. (B) Correlation between the times required for half maximal fibrin polymer formation ($T_{1/2}$ fibrin) and half maximal FXIII-A truncation ($T_{1/2}$ FXIII-A'). Equation for the linear regression line, correlation coefficient, level of significance and the number of investigated plasma samples are indicated on the figure.

$T_{1/2}$ values for FXIII activation in the plasma from Val34Val wild-type individuals, from Val34Leu heterozygotes and from Leu34Leu homozygotes were practically identical (Table 1).

Table 1. The influence of FXIII-A Val34Leu polymorphism on the time required for half maximal activation of FXIII ($T_{1/2}$ FXIII-A') and on the lag time between fibrin formation and FXIII-A truncation ($T_{1/2}$ for FXIII-A' - $T_{1/2}$ for fibrin).

FXIII-A genotype	$T_{1/2}$ for FXIII-A' (s)	$T_{1/2}$ for FXIII-A' – $T_{1/2}$ for fibrin (s)
Val34Val (n=7)	156±11	34.2±7.3
Val34Leu (n=7)	159±29	nd.
Leu34Leu (n=7)	156±14	14.5±4.0*

Means and standard errors are shown in the table. * $p<0.05$ as compared to Val34Val genotype.
nd.: not determined

The results indicate that in whole plasma fibrin polymerization and not FXIII-A Val34Leu polymorphism is the primary determinant for the onset of FXIII activation. However, a closer analysis of the time course of fibrin formation and FXIII activation revealed that even in whole plasma FXIII-A Val34Leu polymorphism is not without effect on the proteolytic activation process (Figure 4). Fibrin formation preceded FXIII activation in all three genotypes, but the lag time between fibrin formation and FXIII-A truncation was significantly longer in the plasma of wild-type individuals than in the plasma of Leu/Leu homozygotes (Figure 4 and Table 1). γ-chain cross-linking starts with the activation of FXIII, but then it slightly precedes it (Figure 4), which is not surprising given that a small amount of activated FXIII is sufficient for full dimerization of γ-chains.

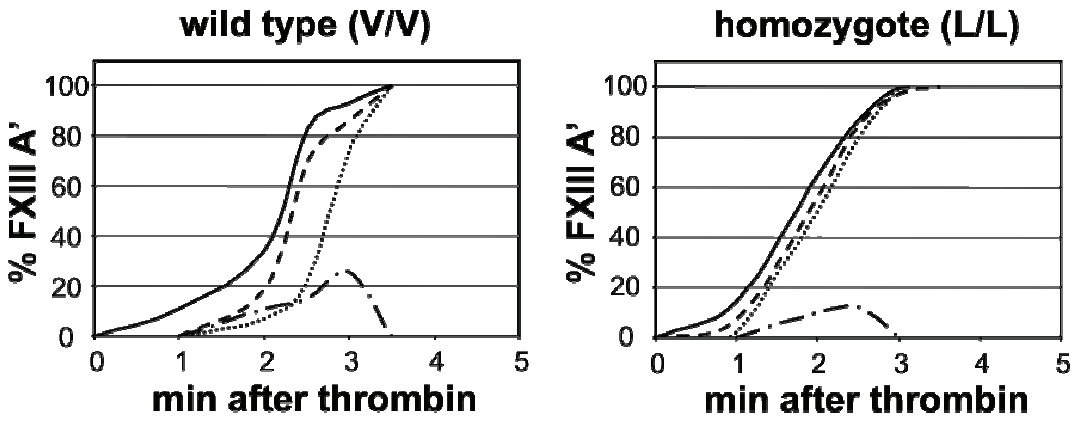

Figure 4. The time course of fibrin polymerization, FXIII-A truncation and fibrin γ-chain cross-linking by FXIIIa following the addition of thrombin to whole plasma.
Representative fibrin samples formed in the plasma from an individual of FXIII-A Val34Val, or Leu34Leu genotype. Samples were removed for analysis in every 20 seconds. Lines represent fibrin formation (————), γ-chain dimerization (- - - - - -), truncation of FXIII-A (••••••••••), non-truncated FXIII-A associated with fibrin (— • — • —). Results are expressed as a percentage of maximal fibrin and γ-chain dimer formation, or as a percentage of FXIII-A' present at full activation.

To provide further evidence for the influence of Val34Leu polymorphism on the thrombin activation of FXIII in plasma, experiments on two FXIII deficient plasma samples, substituted with highly purified FXIII of Val34Val or Leu34Leu genotype, were performed (Figure 5). In these plasma samples everything but FXIII genotype was the same. Although the two plasma samples demonstrated a different time course of fibrin polymerization, the formation of truncated FXIII-A in samples containing FXIII of Val/Val genotype clearly lagged behind the appearance of FXIII-A' in samples containing FXIII of Leu/Leu genotype.

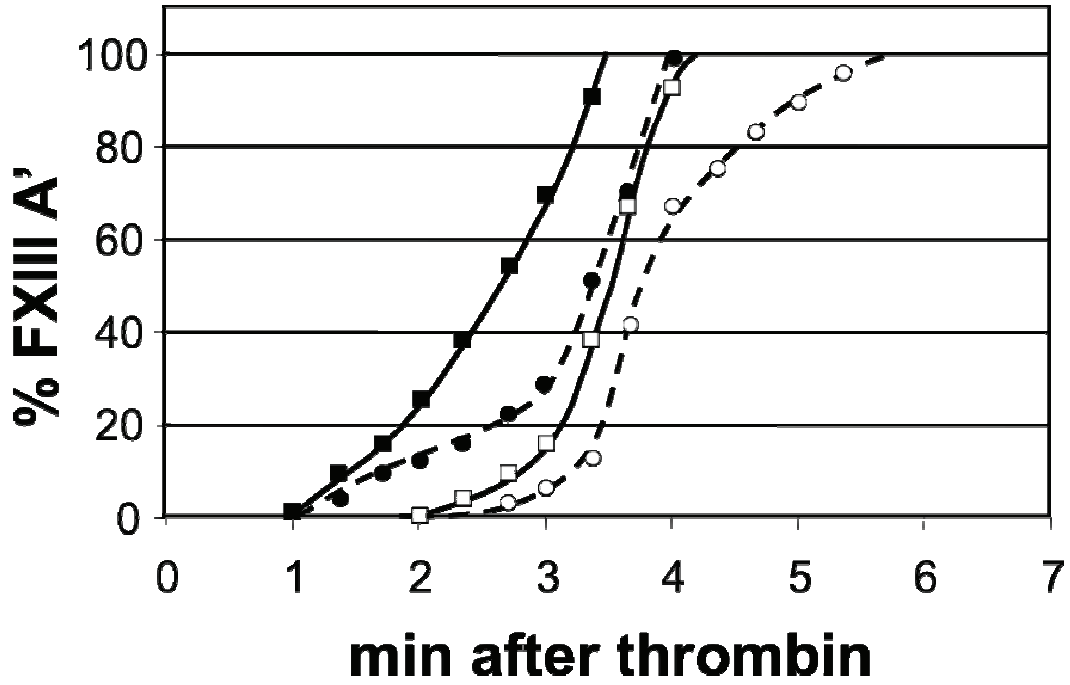

Figure 5. Truncation of FXIII-A by thrombin in two factor deficient plasma samples substituted with highly purified FXIII-A of Val34Val (- - - - - - -) or Leu34Leu (—————) genotype. Sample 1: left side (solid symbols), sample 2: right side (open symbols). Samples were removed for analysis in every 20 seconds. Results are expressed as a percentage of FXIII-A' present at full activation.

Factor XIII levels in PAD patients

Characteristics of subject groups

Table 2 demonstrates the general characteristics of patient and clinical control groups. Patients with PAD were 5.3 years older than subjects in the clinical control group. Diabetes mellitus and smoking was somewhat more frequent among PAD patients than among controls; however the difference did not reach the level of statistical significance. With the exception of homocysteine only marginal differences were detected between the patient and control group in the traditional risk factors. The elevation of total plasma homocysteine concentration in patients with PAD was

highly significant, and it seemed to be related to decreased B12 vitamin levels. Neither FXIII activity nor FXIII A_2B_2 antigen levels of clinical controls differed significantly from the reference interval established in our laboratory (8, 74). Non-adjusted plasma FXIII activity and antigen (FXIII A_2B_2) levels were significantly higher in the patient group.

Adjusted factor XIII levels

Mean FXIII activity and antigen levels adjusted for the respective independently associated parameters were moderately, but significantly higher in the patient than in the clinical control group (Table 3). When the results were analyzed according to gender, in women the presence of PAD was associated with a statistically significant (8-9%) elevation of FXIII activity and antigen levels. In the male PAD subgroup somewhat lower elevations were observed and the difference in FXIII antigen concentrations did not reach the level of statistical significance. No difference in adjusted FXIII levels were found in PAD patients with Fontaine II and Fontaine III stage disease (not shown), and ABPI values did not show significant correlation with FXIII activity or antigen level.

Elevated factor XIII as risk factor of peripheral artery disease

It was also investigated if FXIII levels in the upper tertile (FXIII activity > 120 %, FXIII antigen >25.5 mg/L) represent a risk of PAD as compared to the rest of the patients (Table 4). In males elevated FXIII activity or antigen level did not increase the risk of PAD. In contrast, elevated FXIII activity conferred a more than two-fold, statistically significant risk of PAD on females. FXIII antigen in the upper tertile also represented a two-fold risk of PAD in women, however in this case the P value was somewhat above the limit of statistical significance.

Table 2. Baseline characteristics of clinical controls and patients with peripheral artery disease.

Subject groups	Clinical controls (n = 278)	PAD patients (n = 278)	p value
Gender, (M/F)	173/105	173/105	na
Age, years	59.0 (49.5-68.5)	64.3 (52.1-75.3)	<0.001
Diabetes mellitus, (Y/N)	55/223	69/209	0.15
Ever smoker, (Y/N)	84/194	101/177	0.09
Cholesterol (mmol/L)	5.56 (4.38-6.74)	5.68 (4.46-6.90)	0.24
LDL-C (mmol/L)	3.44 (2.44-4.44)	3.49 (2.43-4.55)	0.52
HDL-C (mmol/L)	1.40 (1.17-1.67)	1.32 (1.11-1.60)	0.006
Apo B (g/L)	1.03 (0.77-1.29)	1.07 (0.78-1.36)	0.07
Apo AI (g/L)	1.62 (1.43-1.86)	1.50 (1.34-1.69)	<0.001
Triglyceride (mmol/L)	1.53 (1.05-2.19)	1.50 (1.08-2.18)	0.99
Lp(a) (mg/L)	139 (99-410)	149 (75-399)	0.29
Fibrinogen (g/L)	3.69 (3.09-4.33)	3.76 (3.22-4.55)	0.32
hsCRP (mg/L)	2.64 (1.28-5.79)	3.03 (1.65-6.36)	0.09
Homocysteine (μmol/L)	12.4 (10.4-15.3)	15.0 (11.5-18.3)	<0.001
Vitamin B12 (pmol/L)	249 (186-342)	202 (150-275)	<0.001
Folic acid (nmol/L)	13.6 (9.7-18.9)	14.4 (10.9-19.0)	0.17
FXIII activity (%)	108 (85-131)	115 (94-136)	<0.001
FXIII antigen (mg/L)	23.4 (18.4-28.4)	24.5 (19.6-29.4)	0.007

Values for fibrinogen, triglyceride, HDL-C, apo AI, hsCRP, homocysteine, folic acid and vitamin B12 are medians with interquartile range in parenthesis, all other continuous variables are means with the interval between mean-SD and mean+SD in parenthesis. na.: non-applicable.

Table 3. Non-adjusted and adjusted factor XIII levels in male and female patients with peripheral artery disease.

		Clinical controls Mean (95% CI)	PAD patients Mean (95% CI)	p value
FXIII activity (%) NA	**total**	108 (105-110)	115 (113-118)	<0.001
	female	109 (105-112)	118 (114-122)	0.001
	male	107 (104-111)	114 (111-117)	0.008
FXIII antigen (mg/L) NA	**total**	23.4 (22.8-23.9)	24.5 (23.9-25.1)	0.007
	female	23.4 (22.5-24.3)	25.2 (24.2-26.2)	0.009
	male	23.3 (22.5-24.1)	24.1 (23.4-24.8)	0.16
FXIII activity (%) A	**total**	107 (105-110)	116 (113-118)	<0.001
	female	108 (105-113)	118 (114-122)	0.001
	male	106 (103-110)	114 (111-118)	0.002
FXIII antigen (mg/L) A	**total**	23.3 (22.8-23.9)	24.4 (23.8-25.0)	0.012
	female	23.4 (22.4-24.4)	25.2 (24.3-26.0)	0.012
	male	23.2 (22.5-24.0)	24.0 (23.2-24.7)	0.18

Values represent non-adjusted (NA) and adjusted (A) mean plasma FXIII activity or antigen (95% confidence interval). A multiple linear regression analysis was performed for FXIII activity and antigen to determine the parameters independently associated with FXIII levels and these parameters were used for adjustment. FXIII activity was adjusted for age, smoking, cholesterol, apo B, HDL-C and vitamin B12, and FXIII antigen values were adjusted for age, smoking, cholesterol, apo B, HDL-C and diabetes mellitus. The significance of differences in mean FXIII values between the clinical control and patient groups were tested by analysis of variance (ANOVA). Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS).

Table 4. The effect of FXIII levels in the upper tertile on the risk of peripheral artery disease in males and females.

	PAD patients versus clinical controls			
	Non-adjusted		Adjusted	
	OR (95% CI)*	**p value**	**OR (95% CI)***	**p value**
FXIII activity				
female	2.341 (1.286-4.261)	0.005	2.316 (1.157-4.635)	0.02
male	1.300 (0.829-2.038)	0.25	1.646 (0.936-2.893)	0.08
FXIII antigen				
female	1.863 (0.947-3.668)	0.07	2.000 (0.943-4.240)	0.07
male	0.871 (0.516-1.470)	0.60	0.743 (0.389-1.418)	0.37

*The risk represented by non-adjusted and adjusted FXIII activity and antigen levels being in the upper tertile, as compared to the rest of the patients, was expressed as the odds ratio (OR) and 95 percent confidence interval. ORs were computed from the corresponding regression coefficient in the logistic regression model. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS).

The effect of FXIII-A Val34Leu genotype on the risk of peripheral artery disease (PAD)

Table 5 demonstrates the frequency of FXIII-A Val34Leu genotypes, Leu34 carriers and Leu34 allele in the group of patients who suffered PAD and in a sex-matched control group. There was no significant difference in genotypes, carrier or allele frequencies between the two groups. Similar negative results were obtained, if the groups were subdivided according to gender and the female and male patient groups were compared with their respective control groups.

The effect of FXIII-A Val34Leu polymorphism on the risk of PAD is shown in Table 6. The odds ratios were calculated against sex matched control groups. No significant effect of Leu34 carriership or the presence of one or two Leu34 alleles was detected. The situation was the same if female or male patients were compared with their respective controls.

Table 5. The distribution of FXIII-A Val34Leu genotypes among patients with peripheral artery disease and clinical controls.

	Clinical controls			**PAD Patients**		
	Total	**Female**	**Male**	**Total**	**Female**	**Male**
Number	278	105	173	278	105	173
Age: median	59	66	55	64	63	65
(IQR)	(51-66)	(62-70)	(48-60)	(55-73)	(54-72)	(56-73)
Val34Leu genotype						
Val/Val	152 (54.7%)	58 (55.2%)	94 (54.3%)	164 (59.0%)	58 (55.2%)	106 (61.3%)
Val/Leu	108 (38.8%)	39 (37.1%)	69 (39.9%)	94 (33.8%)	37 (35.2%)	57 (32.9%)
Leu/Leu	18 (6.5%)	8 (7.6%)	10 (5.8%)	20 (7.2%)	10 (9.5%)	10 (5.8%)
L34 carriers	126 (45.3%)	45 (42.8%)	79 (45.7%)	114 (41.0%)	69 (44.8%)	155 (38.7%)
L34 allele	25.9%	26.2%	25.7%	24.1%	27.1%	22.3%

IQR: inter-quartile range.

Table 6. The effect of FXIII-A Leu34 allele on the risk of peripheral artery disease.

FXIII-A Val34Leu genotype	Total		Female		Male	
	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
Comparison with matched controls						
Leu34 carriers	0.839 (0.599-1.173)	0.304	1.000 (0.580-1.723)	1.000	0.752 (0.490-1.154)	0.192
Val/Leu	0.807 (0.566-1.149)	0.234	0.949 (0.532-0.692)	0.858	0.733 (0.468-1.146)	0.733
Leu/Leu	1.030 (0.525-2.020)	0.932	1.250 (0.461-3.392)	0.661	0.877 (0.354-2.224)	0.798

OR: odds ratio, CI: confidence interval. *ORs were adjusted for age.

The effect of factor XIII A subunit Val34Leu polymorphism on the risk of atherothrombotic ischemic stroke (AIS)

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 non-fatal 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 7). 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.

Table 7. The distribution of FXIII-A Val34Leu genotypes in the population control group.

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

IQR: inter-quartile range.

General charcteristics of the patients surviving AIS

Table 8 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 8. 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.

n.a., non-applicable; IQR, inter-quartile range. *$p<0.001$.

The effect of FXIII-A Val34Leu genotype on the risk of non-fatal ischemic stroke

Table 9 demonstrates the frequency of FXIII-A Val34Leu genotypes, Leu34 carriers and Leu34 allele in the group of patients who suffered non-fatal AIS and in an age and sex-matched control group. There was no significant difference in genotypes, carrier or allele frequencies between the

two groups. Similar negative results were obtained, if the groups were subdivided according to gender and the female and male patient groups were compared with their respective age-matched control groups.

Table 9. The distribution of FXIII-A Val34Leu genotypes among patients with non-fatal atherothrombotic ischemic stroke (AIS) and matched controls.

	Age and sex-matched controls			**Patients with AIS**		
	Total	**Female**	**Male**	**Total**	**Female**	**Male**
Number	496	159	337	496	159	337
Age*	51 (44-62)	47 (41-59)	53 (45-63)	52 (44-65)	47 (41-59)	54 (46-68)
Val34Leu genotype						
Val/Val	272 (54.8%)	93 (52.2%)	189 (56.1%)	274 (55.2%)	91 (57.2%)	183 (54.3%)
Val/Leu	195 (39.4%)	67 (42.1%)	128 (38.0%)	194 (39.1%)	61 (38.4%)	133 (39.5%)
Leu/Leu	29 (5.8%)	9 (5.7%)	20 (5.9%)	28 (5.7%)	7 (4.4%)	21 (6.2%)
L34 carriers	224 (45.1%)	76 (47.8%)	148 (43.9%)	222 (44.7%)	68 (42.8%)	154 (45.7%)
L34 allele	25.5%	26.7%	24.9%	25.2%	23.6%	26.0%

*median (inter-quartile range).

The effect of FXIII-A Val34Leu polymorphism on the risk of non-fatal AIS is shown in Table 10. The odds ratios were calculated against age and sex matched control groups as well as against the whole population control group. No significant effect of Leu34 carriership

or the presence of one or two Leu34 alleles was detected. The situation was the same if female or male patients were compared with their respective controls.

Table 10. The effect of FXIII-A Leu34 allele on the risk of non-fatal atherothrombotic ischemic stroke.

FXIII-A Val34Leu genotype	**Total** **OR (95% CI)**	**Female** **OR (95% CI)**	**Male** **OR (95% CI)**
Comparison with matched controls			
Leu34 carriers	0.984 (0.766-1.264)	0.816 (0.524-1.270)	1.075 (0.793-1.456)
Val/Leu	0.988 (0.761-1.281)	0.830 (0.526-1.311)	1.073 (0.782-1.473)
Leu/Leu	0.958 (0.555-1.654)	0.709 (0.253-1.990)	1.084 (0.569-2.067)
Comparison with the population control group*			
Leu34 carriers	0.993 (0.800-1.233)	0.865 (0.610-1.228)	1.081 (0.822-1.423)
Val/Leu	0.965 (0.771-1.209)	0.858 (0.598-1.233)	1.039 (0.779-1.386)
Leu/Leu	1.188 (0.749-1.884)	0.917 (0.423-1.986)	1.350 (0.726-2.376)

*ORs (odds ratios) were adjusted for sex and age in the total group and for age in female and male groups; CI, confidence interval.

The effect of FXIII-A Val34Leu genotype on the risk of fatal ischemic stroke

The general characteristics of the study group of patients who died of AIS is shown in Table 11. Although there was a statistically significant difference in the age of females and male other characteristics of the study group showed no gender difference.

Table 11. General characteristics of study population with fatal atherothrombotic ischemic stroke (AIS).

	Patients with fatal AIS		
	Total	**Female**	**Male**
Number	316	143	173
Age: median (IQR)	73 (64-81)	76 (78-81)	70 (62-78)*
Hypertension	202 (63.9%)	90 (62.9%)	112 (64.7%)
Hyperlipidemia	173 (54.7 %)	77 (53.8%)	96 (55.5%)
Diabetes mellitus	90 (28.5%)	40 (28%)	50 (28.9%)

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

Due to the high age of patients died of AIS, the median age of sex-matched control group that could be selected from control population was 10 years lower than that of the patient group. The frequency of Val/Val, Val/Leu genotypes, Leu34 carriers and Leu34 allele did not differ significantly between the group of patient with fatal AIS and their respective sex-matched controls (Table 12). However, the frequency of Leu34 homozygotes was significantly higher in the non-divided patient group ($p = 0.007$) and among female patients with fatal AIS ($p = 0.009$)

than in the respective control groups. The difference between the male patient group and the respective control group was not significant.

Table 12. The distribution of FXIII-A Val34Leu genotypes among patients with fatal atherothrombotic ischemic stroke (AIS) and sex-matched controls.

	Sex-matched controls			**Patients with fatal AIS**		
	Total	**Female**	**Male**	**Total**	**Female**	**Male**
Number	316	143	173	316	143	173
Age: median (IQR)	63 (56-72)	67 (60-75)	59 (53-68)	73 (64-81)	76 (78-81)	70 (62-78)
Val34Leu genotype						
Val/Val	186 (58.9%)	80 (58.7%)	102 (59.0%)	177 (56.0%)	77 (53.8%)	100 (57.8%)
Val/Leu	115 (36.4%)	53 (37.1%)	62 (35.8%)	105 (33.2%)	47 (32.9%)	58 (33.5%)
Leu/Leu	15 (4.7%)	6 (4.2%)	9 (5.2%)	34 (10.8%)*	19 (13.3%)**	15 (8.7%)
L34 carriers	130 (41.1%)	59 (41.2%)	71 (41.0%)	139 (44.0%)	66 (46.1%)	73 (42.2%)
L34 allele	22.9%	22.7%	23.1%	27.4%	29.7%	25.4%

* $p = 0.007$, ** $p = 0.009$.

Table 13. The effect of FXIII-A Leu34 allele on the risk of fatal atherothrombotic ischemic stroke.

FXIII-A Val34Leu genotype	**Total** **OR (95% CI)**	**Female** **OR (95% CI)**	**Male** **OR (95% CI)**
Comparison with matched controls			
Leu34 carriers	1.067 (0.763-1.494)	1.260 (0.773-2.053)	0.911 (0.570-1.458)
Val/Leu	0.904 (0.634-1.086)	0.992 (0.588-1.676)	0.821 (0.501-1.345)
Leu/Leu	**2.430 (1.219-4.844)***	**3.825 (1.376-10.628)****	1.667 (0.627-4.427)
Comparison with the population control group			
Leu34 carriers	0.999 (0.732-1.364)	1.298 (0.830-2.029)	0.776 (0.500-1.204)
Val/Leu	0.854 (0.612-1.191)	1.052 (0.649-1.704)	0.709 (0.447-1.126)
Leu/Leu	**2.049 (1.148-3.655)*****	**3.085 (1.393-6.834)******	1.280 (0.544-3.048)

OR, odds ratio; CI, confidence interval. When patient groups were compared with sex-matched control groups ORs were adjusted for age. When patient groups were compared with population control groups ORs were adjusted for sex and age (total group) or for age (females and males). *p = 0.012, **p = 0.010, ***p = 0.015, ****p = 0.005.

Age adjusted ORs were calculated against sex-matched controls and against the population control group (Table 13). Neither Leu34 carriership nor hetrozygousity for the Leu34 allele influenced the risk of fatal AIS significantly in either gender. In contrast, homozygous occurrence of the Leu34 allele significantly increased the risk of fatal AIS in the total patient group. However, this effect was restricted to females, to which gender a more than 3-fold increased risk of fatal AIS was conferred by the presence of two Leu34 alleles.

DISCUSSION

FRET based melting point analysis method for genotyping FXIII-A Val34Leu polymorphism

Our FRET based melting point analysis method was used throughout the study for genotyping FXIII-A Val34Leu polymorphism and the method has been adopted by other laboratories. Two other real-time PCR methods, apyrase-mediated allele-specific primer extension (75) and 5' nuclease assay (76), has also been described for genotyping FXIII-A Val34Leu polymorphism. The results obtained by the assays were compared to those obtained by DNA sequencing on a relatively small number (17 and 20, respectively) of samples. Both methods seem to represent a promising alternative to earlier assays. However, as discrimination among different genotypes by these methods is based on quantitative measures, comparisons with reference methods on a larger number of samples would have been required to confirm their specificity. The real time PCR method presented in this study uses FRET detection and differentiates among genotypes on the basis of a clear difference in the Tm of wild type and mutant allele. With LightCycler the amplification of the target DNA and determination of the genotype are performed in the same closed capillary, limiting the risk of error and contamination considerably. The LightCycler software allows for obtaining a large amount of information from a single run. The assay is specific, reproducible and the total analysis of 31 samples in the LightCycler takes less than 30 min. Its specificity has been confirmed by comparison with the most frequently used PCR-RFLP method and with DNA sequencing on a large number of samples. Since the introduction of the

assay more than 3,000 patients' samples have been tested for Val34Leu polymorphism in our laboratory and in all cases a clear discrimination among the different FXIII-A Val34Leu genotypes could be observed.

Effect of fibrin formation and factor XIII-A subunit Val34Leu polymorphism on the activation of factor XIII

The aim of this study was to determine the relative importance of fibrin formation and FXIII-A Val34Leu polymorphism in controlling the time and the rate of the proteolytic step of FXIII activation and to reveal their interaction in whole plasma. In accordance with earlier reports (77, 78) factor XIII bound to the newly formed fibrin polymer in its native non-truncated form, but with a short lag phase it soon became cleaved by thrombin. The results suggest that at a thrombin concentration that occur in physiological conditions the activation of FXIII takes place exclusively on the surface of fibrin and after its formation FXIIIa remains associated with its substrate. There was a highly significant correlation between the time required for half maximal activation ($T_{1/2}$) for FXIII activation and $T_{1/2}$ for fibrin formation. These results indicate that in whole plasma fibrin polymerization and not FXIII-A Val34Leu polymorphism is the primary determinant for the onset of FXIII activation. However, a closer analysis of the time course of fibrin formation and FXIII activation revealed that even in whole plasma FXIII-A Val34Leu polymorphism is not without effect on the proteolytic activation of FXIII. The lag time between fibrin formation and FXIII-A truncation was significantly longer in the plasma of wild-type individuals than in the plasma of Leu/Leu homozygotes. Further evidence on the influence of Val34Leu polymorphism on the thrombin activation of FXIII in plasma, was provided by

experiments on FXIII deficient plasma samples, substituted with FXIII of Val34Val or Leu34Leu genotype. The proteolytic activation of FXIII-A started at the same time, but proceeded significantly faster in samples containing FXIII of Leu/Leu genotype than in the samples containing FXIII of Val/Val genotype. In conclusion, in whole plasma the onset of FXIII activation is primarily determined by fibrin formation; however, once the activation of FXIII starts, the rate of activation is modulated by Val34Leu polymorphism.

It is not surprising that the primary determinant of the initiation of FXIII activation is the formation and at least partial polymerization of fibrin. It is known that the conversion of fibrinogen into fibrin, i.e. the formation of at least partially polymerized fibrin I and fibrin II greatly enhances the thrombin-catalyzed activation of FXIII. The cleavage of FXIII-A by thrombin in the fibrin I-FXIII complex (kcat/Km = 1.4 x 10^7 M^{-1} sec^{-1}) was 100-fold more efficient than the cleavage of free, uncomplexed FXIII (kcat/Km = 1.4 x 10^5 M^{-1} sec^{-1}) (79). At the same time only a 2.5-fold increase in the rate of FXIII-AP release is conferred by the Leu34 allele to the thrombin induced activation process (46-48). Thus, the onset of fibrin polymerization well overrules any effect of Val34Leu polymorphism and becomes the initiator of FXIII activation. However, the promoting effect of polymeric fibrin is rapidly lost, when catalytically competent FXIIIa is allowed to form (80). When the degree of γ-chain dimerization exceeds approximately 40%, for which minor amount of FXIIIa is sufficient, the promoter effect of fibrin is completely abrogated. This means, that although the role of FXIII Val34Leu polymorhism in the initiation of FXIII activation in whole plasma is negligible, after initiation the rate of the activation is modified by the polymorphism, and the release of FXIII-AP proceeds significantly faster in the case of Leu34 allele. It is interesting to speculate if such a difference in the rate of activation has any physiological significance. It has been shown by Schröder and

Kohler (53) that the higher rate of proteolytic truncation of Leu34 FXIII-A resulted in accelerated cross-linking of α_2PI to fibrin α-chain. The formation of this heterodimer is a fast process, only slightly lags behind γ-chain dimerization, i.e. Leu34 FXIII-A provides an earlier protection of newly formed fibrin against the prompt elimination by the fibrinolytic system.

It remains to be seen if such modification of the rate of FXIII activation by FXIII-A Val34Leu polymorphism can be related to the possible protective effect of the Leu34 allele against coronary artery disease and venous thromboembolism. The fact that cross-linking of fibrin by FXIIIa down-regulates the adhesion of platelets to fibrin (81, 82) might offer the following hypothesis: earlier activation of Leu34 FXIII-A would result in earlier cross-linking of fibrin in the growing thrombus, that would down-regulate platelet adhesion and inhibit further thrombus growth. The influence of FXIII-A Val34Leu polymorphism on fibrin structure, making it more prone to fibrinolysis, is another alternative hypothesis (48). This mechanism seem to be effective only at high fibrinogen concentration (55). The biochemistry of this effect of Val34Leu polymorphism is still to be revealed.

Factor XIII and the risk of peripheral artery disease

FXIII levels were elevated in patients with PAD, and this was more pronounced in women than in men. The extent of FXIII elevation did not correlate with the severity of PAD. One may conclude from these results that the existence of PAD and not the difference in its severity is associated with the elevation in FXIII levels. In a small (n=50) early study elevation of non-adjusted FXIII levels has also been observed in PAD patients (73), however in this case the

patients were not characterized and differences according to gender and severity of the disease were not analyzed.

The reason for the elevation of FXIII levels in PAD is not known. Plasma FXIII is a tetrameric complex of two potentially active A and two inhibitor/carrier B subunits (FXIII-A and FXIII-B). FXIII-B is in excess to FXIII-A, i.e., the actual amount of FXIII A_2B_2 complex is determined by the amount of FXIII-A. Megakaryocytes are a major source of FXIII-A synthesis and circulating platelets contain a huge amount of FXIII-A. Although platelet FXIII-A is retained during platelet activation, platelets may become damaged when pressed through nearly occluded arteries and release their FXIII-A content into the circulation. Such a mechanism could contribute to the increase of FXIII level in patients with PAD.

Elevated FXIII level conferred a more than two-fold, statistically significant risk of PAD on females. Several pieces of evidence suggest that impaired fibrinolysis caused by elevated PAI-1 level and decreased tPA activity increases the risk of PAD (83-87). Factor XIII is a major regulator of fibrinolysis; it strengthens fibrin clot and makes it more resistant to shear forces and to fibrinolysis. At elevated FXIII levels this mechanism could be more forceful and in PAD, together with the elevation of α_2PI level (88), could participate in impairing the fibrinolytic potential. In addition, FXIIIa has also been shown to enhance migration and proliferation of smooth muscle cells and monocytes (89, 90), two processes, which have also been implicated in the progression of atherosclerosis. It remains to be seen, why the effect of elevated FXIII level is more prominent in women.

It is known that the onset of acute complications of atherothrombotic disease occurs at older ages in women than in men, but even after adjustment for age, women have poorer outcome (91, 92). Although the mechanism has not been revealed, it seems that in females

hemostatic risk factors are more important determinants of atherothrombotic diseases than in males. In the case of CAD the poorer outcome has been connected to gender-related differences in hemostatic risk factors including fibrinogen and PAI-1 (93-97). It has also been shown that elevated FXIII levels represent a risk factor of MI only in women (64). This result together with our finding suggests that elevated FXIII is a gender-specific risk factor of these two atherothrombotic diseases and supports the suggestion that in atherothrombotic diseases the clotting/fibrinolytic system plays a role more prominent in females than in males (98).

Only a single report was found on the association of FXIII-A Val34Leu polymorphism and PAD (99). In this study, like in our study, the polymorphism did not influence the occurrence of PAD, but there was a later onset of PAD in Leu/Leu homozygotes (mean age 67.3 years) than in Val/Val wild type individuals (mean age: 64.1 years). The lack of data on the association of FXIII-A Val34Leu polymorphism and PAD warrants further investigations.

Atherothrombotic Ischemic Stroke (AIS) and FXIII A Val34Leu polymorphism

We were able to collect 13 publications in the literature on the relationship of FXIII-A Val34Leu polymorphism and the risk of IS (67, 100-111). Most of the studies (100, 101, 104-106, 109-111) as well as an interim mini-metaanalysis (112), demonstrated the lack of association of FXIII-A Val34Leu genotypes with the risk of IS, although in a few reports protection against IS (102, 103, 108) or increased risk of IS (67, 107) was associated with the polymorphism. In nine of these publications AIS and cardioembolic IS cases were considered as a common group. However, due to the different pathomechanism of the two types of IS, the same genetic or environmental factors might exert different, occasionally opposing, effects on the risk of AIS and cardioembolic IS. Thus, the results obtained with the common group of patients might obscure

results that could have been obtained by separate risk assessment. Only in 4 published smaller studies was AIS separated from cardioembolic stroke in the analysis of the risk associated with FXIII-A Val34Leu polymorphism (102, 104, 105, 108). In two of these studies (n=31 and n=121) the polymorphism was found neutral (104, 105). In another study involving 105 AIS patients an OR of 0.63 (CI: 0.37-1.10) suggested a protective effect against AIS, however it did not reach statistical significance (102). In two cases the effect of FXIII-A Val34Leu polymorphism on the risk of small vessel disease stroke was investigated. No protective effect was found by Endler et al. (n=159) (105), while in a small study (n=66) the Val/Val genotype conferred a 2.1-fold increased risk of stroke (CI: 1.1- 3.9)(108). To eliminate the possibility of false negative result due to small sample size (113), our study involved 496 patients, and it is far more the largest study in which the effect of FXIII-A Val34Leu polymorphism on the risk of AIS, separately from cardioembolic IS was investigated. Comparison with both a sex and age matched control group and with a general population control group, which reflects the general occurrence of the polymorphism in the population, could not detect any protective effect of Leu34 carriership and homozygous or heterozygous Leu34 variants against the risk of AIS.

The results obtained with CAD (64) and PAD (see above) seemed to justify the gender specific analysis of the effect of FXIII-A Val34Leu polymorphism on the risk of AIS. Two former studies have been published on the association of FXIII-A Val34Leu polymorphism and IS in females, however, here again, AIS and cardioembolic stroke were not analyzed separately. Reiner et al. reported a nearly 4-fold increase of IS in young women carrying two copies of the Leu34 allele (67). However, this study involved only 36 cases. In a larger study (n = 190) on young women no significant effect of Val34Leu polymorphism on the risk of IS could be

revealed (109). In our study on AIS survivor patients the polymorphism failed to exert a significant effect on the risk of AIS in either gender.

In summary, FXIII Val34Leu polymorphism does not seem to be a significant protective or risk factor of AIS, although genetic and environmental factors might modulate this picture and might be responsible for some of the contradictory findings. AS discussed earlier, the modulatory effect of insulin resistance and fibrinogen level on the effect of FXIII-A Val34Leu polymorphism has been revealed in CAD patients (68, 71, 72, 114). Such interactions still await to be investigated in the case of AIS. The only study in this respect concerned the interactive effect of fibrinogen level and FXIII-A Val34Leu polymorphism on the outcome of thrombolytic therapy of IS (71). Patients with wild type FXIII-A and low fibrinogen level displayed the best clinical outcome; while carriers of the Leu34 allele having high fibrinogen level showed almost no clinical response.

To our knowledge this is the first study concerning the effect of Val34Leu polymorphism on the risk of fatal AIS. In our study neither Leu34 carriership nor Val34Leu heterozygousity influenced the risk of fatal AIS significantly. In contrast, the presence of double Leu34 alleles conferred an approximately three-fold risk of AIS with fatal outcome on women, but not on men. This interesting gender-specific effect suggests that, although homozygous form of Leu34 allele did not influence the risk of the onset of AIS, it increased the risk of more severe consequences in women. The fact that the OR in heterozygous women was close to 1.0 excludes a gene dosage effect.

The findings reported in this study raise two questions. 1/ Why homozygous presence of Leu34 allele increases the risk of fatal IS? 2/ Why is the high risk of fatal AIS conferred only on women? The Leu allele that confers protection against MI has been shown to be a risk factor of

primary intracerebral hemorrhage (100, 103). The fatal outcome of AIS in many cases is due to hemorrhagic transformation of AIS. Following this line it might be that homozygous form of FXIII-A Val34Leu polymorphism increases the risk of hemorrhagic complication of acute AIS. As discussed above the increased severity of acute events in females suffering of CAD has been connected to hemostatic risk factors. Our results on the gender-specific association of FXIII-A Val34Leu polymorphism and fatal AIS support such hypothesis. However, the biochemical background of the increased sensitivity of women to hemostatic risk factors remains to be explored.

SUMMARY

A new method based on real time PCR with fluorescence resonance energy transfer (FRET) detection and melting curve analysis was developed for the detection of factor XIII A subunit (FXIII-A) Val34Leu polymorphism. The rapid, simple method is well applicable for large-scale analysis. The results with this method showed 100% coincidence with those obtained by the traditional PCR-RFLP assay and fluorescent DNA sequencing.

In plasma the time of thrombin induced fibrin formation closely correlated with the time of FXIII activation, while there was no significant correlation between the onset of FXIII activation and FXIII-A Val34Leu genotype. However, in the case of Leu34 variant the proteolytic activation of FXIII proceeded significantly faster than in the case of Val34 variant. The results suggest that in whole plasma the onset of FXIII activation is determined by fibrin formation, while the rate of activation is modulated by Val34Leu polymorphism.

FXIII levels were elevated in patients with peripheral artery disease (PAD) and the elevation was more evident in females than in males. The severity of PAD did not show significant correlation with FXIII levels. FXIII levels in the upper tertile conferred a 2-2.3-fold increased risk of PAD to females, but not to males, i.e., elevated FXIII could be considered a gender specific risk factor of PAD. FXIII-A Val34Leu polymorphism did not influence the risk of PAD.

No association was revealed between the risk of non-fatal atherothrombotic ischemic stroke (AIS) and FXIII-A Val34Leu genotype. In contrast, in females homozygous presentation of Leu34 allele represented a more than 3.0-fold increased risk of AIS with fatal outcome. FXIII-A Val34Leu polymorphism does not influence the occurrence of AIS, but has a gender specific effect on the severity of its outcome.

ÖSSZEFOGLALÁS

Egy új, valós idejű polimeráz lánc reakción (PCR) rekción alapuló, fluoreszcens rezonancia energia transzfer detektálást és olvadáspont analízist használó módszert fejlesztettünk ki a XIII-as factor A alegység (FXIII-A) Val34Leu polimorfizmusának detektálására. A gyors, egyszerű módszer jól használható nagy számú minta analízisére. A hagyományos RFLP módszerrel, a fluoreszcens DNS szekvenálással és az új módszerrel kapott eredmények 100%-ban megegyeztek.

A plazmában a FXIII aktiváció jól korrelált a trombin indukálta fibrin képződéssel, de nem korrelált a FXIII-A Val34leu genotípussal. Ugyanakkor, a Leu34 variáns esetében a FXIII proteolítikus aktivációja szignifikánsan gyorsabb volt mint a Val34 variáns esetében. Az eredmények azt mutatják, hogy plazmában a FXIII aktivációjának kezdetét a fibrin képződés határozza meg, míg a FXIII-A Val34Leu polimorfizmus az aktiváció sebességét befolyásolja.

Perifériás artériás betegségben (PAB) a FXIII szintek szignifikánsan emelkedtek, az emelkedés nőknél kifejezettebb volt mint férfiaknál. A PAB súlyossága és a FXIII szintek emelkedése között nem volt összefüggés. A felső harmadba eső FXIII szintek nőknél a PAB rizikóját 2-2.3-szorosára fokozták, férfiak esetében nem lehetett ilyen hatást kimutatni. Ennek alapján az emelkedett FXIII szint a PAB nemre specifikus rizikó faktorának tekinthető. A FXIII-A Val34Leu polimorfizmus nem volt hatással a PAB előfordulásának gyakoriságára.

A FXIII-A Val34Leu polimorfizmus és a nem halálos aterotrombotikus isémiás stroke (AIS) előfordulásának gyakorisága között sem volt szignifikáns összefüggés. Ezzel szemben, nőkben a Leu34 homozigótaság több mint 3-szorosára növelte a halálos kimenetelú AIS rizikóját. A FXIII-A Val34Leu polimorfizmus tehát nincs hatással az AIS bekövetkeztének gyakoriságára, viszont nemre specifikus módon befolyásolja az AIS kimenetelének súlyosságát.

REFERENCES:

1. Muszbek L, Yee VC, Hevessy Z. Blood coagulation factor XIII: structure and function. Thromb Res. 1999 Jun 1;94(5):271-305.

2. Greenberg CS, Sane DC, Lai T-S. Factor XIII and fibrin stabilization. In: Colman RW, Clowes AW, Goldhaber SZ, Marder VJ, George JN, eds. Hemostasis and Thrombosis. 4th ed. Philadelphia: Lippincott Williams and Wilkins, 2006:317-34.

3. Muszbek L, Bagoly Z, Bereczky Z, Katona E. The involvement of blood coagulation factor XIII in fibrinolysis and thrombosis. Cardiovasc Hematol Agents Med Chem. 2008 Jul;6(3):190-205.

4. Luscher EF. [Fibrin-stabilizing factor from thrombocytes.]. Schweiz Med Wochenschr. 1957 Oct 2;87(39-40):1220-1.

5. Kiesselbach TH, Wagner RH. Demonstration of factor XIII in human megakaryocytes by a fluorescent antibody technique. Ann N Y Acad Sci. 1972 Dec 8;202:318-28.

6. Muszbek L, Adany R, Mikkola H. Novel aspects of blood coagulation factor XIII. I. Structure, distribution, activation, and function. Crit Rev Clin Lab Sci. 1996 Oct;33(5):357-421.

7. Adany R, Belkin A, Vasilevskaya T, Muszbek L. Identification of blood coagulation factor XIII in human peritoneal macrophages. Eur J Cell Biol. 1985 Jul;38(1):171-3.

8. Katona E, Haramura G, Karpati L, Fachet J, Muszbek L. A simple, quick one-step ELISA assay for the determination of complex plasma factor XIII (A2B2). Thromb Haemost. 2000 Feb;83(2):268-73.

9. Yee VC, Pedersen LC, Le Trong I, Bishop PD, Stenkamp RE, Teller DC. Three-dimensional structure of a transglutaminase: human blood coagulation factor XIII. Proc Natl Acad Sci U S A. 1994 Jul 19;91(15):7296-300.

10. Muszbek L, Bereczky Z, Bagoly Z, Komáromi I, Katona E. Factor XIII: a coagulation factor with multiple plasmatic and cellular functions. Physiol Rev. 2010;accepted.

11. Bereczky Z, Katona E, Muszbek L. Fibrin stabilization (factor XIII), fibrin structure and thrombosis. Pathophysiol Haemost Thromb. 2003 Sep-2004 Dec;33(5-6):430-7.

12. Shemirani AH, Haramura G, Bagoly Z, Muszbek L. The combined effect of fibrin formation and factor XIII A subunit Val34Leu polymorphism on the activation of factor XIII in whole plasma. Biochim Biophys Acta. 2006 Aug;1764(8):1420-3.

13. Karimi M, Bereczky Z, Cohan N, Muszbek L. Factor XIII Deficiency. Semin Thromb Hemost. 2009 Jun;35(4):426-38.

14. Sakata Y, Aoki N. Cross-linking of alpha 2-plasmin inhibitor to fibrin by fibrin-stabilizing factor. J Clin Invest. 1980 Feb;65(2):290-7.

15. Lorand L, Jacobsen A. Accelerated lysis of blood clots. Nature. 1962 Sep 1;195:911-2.

16. Gaffney PJ, Whitaker AN. Fibrin crosslinks and lysis rates. Thromb Res. 1979 Jan;14(1):85-94.

17. Mosesson MW, Siebenlist KR, Meh DA. The structure and biological features of fibrinogen and fibrin. Ann N Y Acad Sci. 2001;936:11-30.

18. Mosesson MW. Fibrinogen gamma chain functions. J Thromb Haemost. 2003 Feb;1(2):231-8.

19. Mosesson MW, Siebenlist KR, Amrani DL, DiOrio JP. Identification of covalently linked trimeric and tetrameric D domains in crosslinked fibrin. Proc Natl Acad Sci U S A. 1989 Feb;86(4):1113-7.

20. Shainoff JR, Urbanic DA, DiBello PM. Immunoelectrophoretic characterizations of the cross-linking of fibrinogen and fibrin by factor XIIIa and tissue transglutaminase. Identification of a rapid mode of hybrid alpha-/gamma-chain cross-linking that is promoted by the gamma-chain cross-linking. J Biol Chem. 1991 Apr 5;266(10):6429-37.

21. Siebenlist KR, Meh DA, Mosesson MW. Protransglutaminase (factor XIII) mediated crosslinking of fibrinogen and fibrin. Thromb Haemost. 2001 Nov;86(5):1221-8.

22. Francis RT, McDonagh J, Mann KG. Factor V is a substrate for the transamidase factor XIIIa. J Biol Chem. 1986 Jul 25;261(21):9787-92.

23. Bendixen E, Borth W, Harpel PC. Transglutaminases catalyze cross-linking of plasminogen to fibronectin and human endothelial cells. J Biol Chem. 1993 Oct 15;268(29):21962-7.

24. Borth W, Chang V, Bishop P, Harpel PC. Lipoprotein (a) is a substrate for factor XIIIa and tissue transglutaminase. J Biol Chem. 1991 Sep 25;266(27):18149-53.

25. Valnickova Z, Enghild JJ. Human procarboxypeptidase U, or thrombin-activable fibrinolysis inhibitor, is a substrate for transglutaminases. Evidence for transglutaminase-catalyzed cross-linking to fibrin. J Biol Chem. 1998 Oct 16;273(42):27220-4.

26. Kanaide H, Shainoff JR. Cross-linking of fibrinogen and fibrin by fibrin-stablizing factor (factor XIIIa). J Lab Clin Med. 1975 Apr;85(4):574-97.

27. Mosher DF, Schad PE, Vann JM. Cross-linking of collagen and fibronectin by factor XIIIa. Localization of participating glutaminyl residues to a tryptic fragment of fibronectin. J Biol Chem. 1980 Feb 10;255(3):1181-8.

28. Cho J, Mosher DF. Enhancement of thrombogenesis by plasma fibronectin cross-linked to fibrin and assembled in platelet thrombi. Blood. 2006 May 1;107(9):3555-63.

29. Gupta M, Greenberg CS, Eckman DM, Sane DC. Arterial vimentin is a transglutaminase substrate: a link between vasomotor activity and remodeling? J Vasc Res. 2007;44(5):339-44.

30. Bale MD, Westrick LG, Mosher DF. Incorporation of thrombospondin into fibrin clots. J Biol Chem. 1985 Jun 25;260(12):7502-8.

31. Cohen I, Young-Bandala L, Blankenberg TA, Siefring GE, Jr., Bruner-Lorand J. Fibrinoligase-catalyzed cross-linking of myosin from platelet and skeletal muscle. Arch Biochem Biophys. 1979 Jan;192(1):100-11.

32. Asijee GM, Muszbek L, Kappelmayer J, Polgar J, Horvath A, Sturk A. Platelet vinculin: a substrate of activated factor XIII. Biochim Biophys Acta. 1988 Jun 13;954(3):303-8.

33. Cohen I, Blankenberg TA, Borden D, Kahn DR, Veis A. Factor XIIIa-catalyzed cross-linking of platelet and muscle actin. Regulation by nucleotides. Biochim Biophys Acta. 1980 Mar 20;628(3):365-75.

34. Dardik R, Loscalzo J, Eskaraev R, Inbal A. Molecular mechanisms underlying the proangiogenic effect of factor XIII. Arterioscler Thromb Vasc Biol. 2005 Mar;25(3):526-32.

35. AbdAlla S, Lother H, Langer A, el Faramawy Y, Quitterer U. Factor XIIIA transglutaminase crosslinks AT1 receptor dimers of monocytes at the onset of atherosclerosis. Cell. 2004 Oct 29;119(3):343-54.

36. Inbal A, Lubetsky A, Krapp T, Castel D, Shaish A, Dickneitte G, et al. Impaired wound healing in factor XIII deficient mice. Thromb Haemost. 2005 Aug;94(2):432-7.

37. Dardik R, Loscalzo J, Inbal A. Factor XIII (FXIII) and angiogenesis. J Thromb Haemost. 2006 Jan;4(1):19-25.

38. Inbal A, Muszbek L. Coagulation factor deficiencies and pregnancy loss. Semin Thromb Hemost. 2003 Apr;29(2):171-4.

39. Ichinose A, Davie EW. Characterization of the gene for the a subunit of human factor XIII (plasma transglutaminase), a blood coagulation factor. Proc Natl Acad Sci U S A. 1988 Aug;85(16):5829-33.

40. Board PG, Webb GC, McKee J, Ichinose A. Localization of the coagulation factor XIII A subunit gene (F13A) to chromosome bands 6p24----p25. Cytogenet Cell Genet. 1988;48(1):25-7.

41. Board PG. Genetic polymorphism of the B subunit of human coagulation factor XIII. Am J Hum Genet. 1980 May;32(3):348-53.

42. Lockwood CR, Frayling TM. Combining genome and mouse knockout expression data to highlight binding sites for the transcription factor HNF1alpha. In Silico Biol. 2003;3(1-2):57-70.

43. Ichinose A, Bottenus RE, Davie EW. Structure of transglutaminases. J Biol Chem. 1990 Aug 15;265(23):13411-4.

44. Mikkola H, Syrjala M, Rasi V, Vahtera E, Hamalainen E, Peltonen L, et al. Deficiency in the A-subunit of coagulation factor XIII: two novel point mutations demonstrate different effects on transcript levels. Blood. 1994 Jul 15;84(2):517-25.

45. Muszbek L. Deficiency causing mutations and common polymorphisms in the factor XIII-A gene. Thromb Haemost. 2000 Oct;84(4):524-7.

46. Balogh I, Szoke G, Karpati L, Wartiovaara U, Katona E, Komaromi I, et al. Val34Leu polymorphism of plasma factor XIII: biochemistry and epidemiology in familial thrombophilia. Blood. 2000 Oct 1;96(7):2479-86.

47. Wartiovaara U, Mikkola H, Szoke G, Haramura G, Karpati L, Balogh I, et al. Effect of Val34Leu polymorphism on the activation of the coagulation factor XIII-A. Thromb Haemost. 2000 Oct;84(4):595-600.

48. Ariens RA, Philippou H, Nagaswami C, Weisel JW, Lane DA, Grant PJ. The factor XIII V34L polymorphism accelerates thrombin activation of factor XIII and affects cross-linked fibrin structure. Blood. 2000 Aug 1;96(3):988-95.

49. Trumbo TA, Maurer MC. Examining thrombin hydrolysis of the factor XIII activation peptide segment leads to a proposal for explaining the cardioprotective effects observed with the factor XIII V34L mutation. J Biol Chem. 2000 Jul 7;275(27):20627-31.

50. Trumbo TA, Maurer MC. Thrombin hydrolysis of V29F and V34L mutants of factor XIII (28-41) reveals roles of the P(9) and P(4) positions in factor XIII activation. Biochemistry. 2002 Feb 26;41(8):2859-68.

51. Trumbo TA, Maurer MC. V34I and V34A substitutions within the factor XIII activation peptide segment (28-41) affect interactions with the thrombin active site. Thromb Haemost. 2003 Apr;89(4):647-53.

52. de Lange M, Andrew T, Snieder H, Ge D, Futers TS, Standeven K, et al. Joint linkage and association of six single-nucleotide polymorphisms in the factor XIII-A subunit gene point to V34L as the main functional locus. Arterioscler Thromb Vasc Biol. 2006 Aug;26(8):1914-9.

53. Schroder V, Kohler HP. Effect of factor XIII Val34Leu on alpha2-antiplasmin incorporation into fibrin. Thromb Haemost. 2000 Dec;84(6):1128-30.

54. Lee IH, Chung SI, Lee SY. Effects of Val34Leu and Val35Leu polymorphism on the enzyme activity of the coagulation factor XIII-A. Exp Mol Med. 2002 Nov 30;34(5):385-90.

55. Lim BC, Ariens RA, Carter AM, Weisel JW, Grant PJ. Genetic regulation of fibrin structure and function: complex gene-environment interactions may modulate vascular risk. Lancet. 2003 Apr 26;361(9367):1424-31.

56. Anwar R, Gallivan L, Edmonds SD, Markham AF. Genotype/phenotype correlations for coagulation factor XIII: specific normal polymorphisms are associated with high or low factor XIII specific activity. Blood. 1999 Feb 1;93(3):897-905.

57. Gallivan L, Markham AF, Anwar R. The Leu564 factor XIIIA variant results in significantly lower plasma factor XIII levels than the Pro564 variant. Thromb Haemost. 1999 Oct;82(4):1368-70.

58. Curtis CG, Brown KL, Credo RB, Domanik RA, Gray A, Stenberg P, et al. Calcium-dependent unmasking of active center cysteine during activation of fibrin stabilizing factor. Biochemistry. 1974 Aug 27;13(18):3774-80.

59. Komanasin N, Catto AJ, Futers TS, van Hylckama Vlieg A, Rosendaal FR, Ariens RA. A novel polymorphism in the factor XIII B-subunit (His95Arg): relationship to subunit dissociation and venous thrombosis. J Thromb Haemost. 2005 Nov;3(11):2487-96.

60. Ryan AW, Hughes DA, Tang K, Kelleher DP, Ryan T, McManus R, et al. Natural selection and the molecular basis of electrophoretic variation at the coagulation F13B locus. Eur J Hum Genet. 2009 Feb;17(2):219-27.

61. Iwata H, Kitano T, Umetsu K, Yuasa I, Yamazaki K, Kemkes-Matthes B, et al. Distinct C-terminus of the B subunit of factor XIII in a population-associated major phenotype: the first

case of complete allele-specific alternative splicing products in the coagulation and fibrinolytic systems. J Thromb Haemost. 2009 Jul;7(7):1084-91.

62. Duckert F, Jung E, Shmerling DH. A hitherto undescribed congenital haemorrhagic diathesis probably due to fibrin stabilizing factor deficiency. Thromb Diath Haemorrh. 1960 Dec 15;5:179-86.

63. Muszbek L, Bereczky Z, Bagoly Z, Shemirani AH, Katona E. Factor XIII and atherothrombotic diseases. Semin Thromb Hemost. 2010 Feb;36(1):18-33.

64. Bereczky Z, Balogh E, Katona E, Czuriga I, Edes I, Muszbek L. Elevated factor XIII level and the risk of myocardial infarction in women. Haematologica. 2007 Feb;92(2):287-8.

65. Kohler HP, Stickland MH, Ossei-Gerning N, Carter A, Mikkola H, Grant PJ. Association of a common polymorphism in the factor XIII gene with myocardial infarction. Thromb Haemost. 1998 Jan;79(1):8-13.

66. Voko Z, Bereczky Z, Katona E, Adany R, Muszbek L. Factor XIII Val34Leu variant protects against coronary artery disease. A meta-analysis. Thromb Haemost. 2007 Mar;97(3):458-63.

67. Reiner AP, Frank MB, Schwartz SM, Linenberger ML, Longstreth WT, Teramura G, et al. Coagulation factor XIII polymorphisms and the risk of myocardial infarction and ischaemic stroke in young women. Br J Haematol. 2002 Feb;116(2):376-82.

68. Kohler HP, Mansfield MW, Clark PS, Grant PJ. Interaction between insulin resistance and factor XIII Val34Leu in patients with coronary artery disease. Thromb Haemost. 1999 Sep;82(3):1202-3.

69. Warner D, Mansfield MW, Grant PJ. Coagulation factor XIII and cardiovascular disease in UK Asian patients undergoing coronary angiography. Thromb Haemost. 2001 Mar;85(3):408-11.

70. Collet JP, Park D, Lesty C, Soria J, Soria C, Montalescot G, et al. Influence of fibrin network conformation and fibrin fiber diameter on fibrinolysis speed: dynamic and structural approaches by confocal microscopy. Arterioscler Thromb Vasc Biol. 2000 May;20(5):1354-61.

71. Boekholdt SM, Sandhu MS, Wareham NJ, Luben R, Reitsma PH, Khaw KT. Fibrinogen plasma levels modify the association between the factor XIII Val34Leu variant and risk of coronary artery disease: the EPIC-Norfolk prospective population study. J Thromb Haemost. 2006 Oct;4(10):2204-9.

72. Bereczky Z, Balogh E, Katona E, Pocsai Z, Czuriga I, Szeles G, et al. Modulation of the risk of coronary sclerosis/myocardial infarction by the interaction between factor XIII subunit A Val34Leu polymorphism and fibrinogen concentration in the high risk Hungarian population. Thromb Res. 2007;120(4):567-73.

73. Kloczko J, Wojtukiewicz M, Bielawiec M, Zuch A. Alterations of haemostasis parameters with special reference to fibrin stabilization, factor XIII and fibronectin in patients with obliterative atherosclerosis. Thromb Res. 1988 Sep 15;51(6):575-81.

74. Karpati L, Penke B, Katona E, Balogh I, Vamosi G, Muszbek L. A modified, optimized kinetic photometric assay for the determination of blood coagulation factor XIII activity in plasma. Clin Chem. 2000 Dec;46(12):1946-55.

75. O'Meara D, Ahmadian A, Odeberg J, Lundeberg J. SNP typing by apyrase-mediated allele-specific primer extension on DNA microarrays. Nucleic Acids Res. 2002 Aug 1;30(15):e75.

76. Tjarnlund A, Andersson M, Jood K, Ladenvall P, Jern C. Rapid genotyping of haemostatic gene polymorphisms using the 5' nuclease assay. Thromb Haemost. 2003 May;89(5):936-42.

77. Greenberg CS, Miraglia CC, Rickles FR, Shuman MA. Cleavage of blood coagulation factor XIII and fibrinogen by thrombin during in vitro clotting. J Clin Invest. 1985 May;75(5):1463-70.

78. Brummel KE, Butenas S, Mann KG. An integrated study of fibrinogen during blood coagulation. J Biol Chem. 1999 Aug 6;274(32):22862-70.

79. Naski MC, Lorand L, Shafer JA. Characterization of the kinetic pathway for fibrin promotion of alpha-thrombin-catalyzed activation of plasma factor XIII. Biochemistry. 1991 Jan 29;30(4):934-41.

80. Lewis SD, Janus TJ, Lorand L, Shafer JA. Regulation of formation of factor XIIIa by its fibrin substrates. Biochemistry. 1985 Nov 19;24(24):6772-7.

81. Rubens FD, Perry DW, Hatton MW, Bishop PD, Packham MA, Kinlough-Rathbone RL. Platelet accumulation on fibrin-coated polyethylene: role of platelet activation and factor XIII. Thromb Haemost. 1995 May;73(5):850-6.

82. Skarja GA, Brash JL, Bishop P, Woodhouse KA. Protein and platelet interactions with thermally denatured fibrinogen and cross-linked fibrin coated surfaces. Biomaterials. 1998 Dec;19(23):2129-38.

83. Makin A, Silverman SH, Lip GY. Peripheral vascular disease and Virchow's triad for thrombogenesis. QJM. 2002 Apr;95(4):199-210.

84. Paraskevas KI, Baker DM, Vrentzos GE, Mikhailidis DP. The role of fibrinogen and fibrinolysis in peripheral arterial disease. Thromb Res. 2008;122(1):1-12.

85. Lapolla A, Piarulli F, Sartore G, Rossetti C, Martano L, Carraro P, et al. Peripheral artery disease in type 2 diabetes: the role of fibrinolysis. Thromb Haemost. 2003 Jan;89(1):91-6.

86. Koksch M, Zeiger F, Wittig K, Pfeiffer D, Ruehlmann C. Haemostatic derangement in advanced peripheral occlusive arterial disease. Int Angiol. 1999 Dec;18(4):256-62.

87. Boneu B, Leger P, Arnaud C. Haemostatic system activation and prediction of vascular events in patients presenting with stable peripheral arterial disease of moderate severity. Royat Study Group. Blood Coagul Fibrinolysis. 1998 Mar;9(2):129-35.

88. Christe M, Delley A, Marbet GA, Biland L, Duckert F. Fibrinogen, factor VIII related antigen, antithrombin III and alpha 2-antiplasmin in peripheral arterial disease. Thromb Haemost. 1984 Dec 29;52(3):240-2.

89. Naito M, Nomura H, Iguchi A, Thompson WD, Smith EB. Effect of crosslinking by factor XIIIa on the migration of vascular smooth muscle cells into fibrin gels. Thromb Res. 1998 May 1;90(3):111-6.

90. Dardik R, Krapp T, Rosenthal E, Loscalzo J, Inbal A. Effect of FXIII on monocyte and fibroblast function. Cell Physiol Biochem. 2007;19(1-4):113-20.

91. Glader EL, Stegmayr B, Norrving B, Terent A, Hulter-Asberg K, Wester PO, et al. Sex differences in management and outcome after stroke: a Swedish national perspective. Stroke. 2003 Aug;34(8):1970-5.

92. Di Carlo A, Lamassa M, Baldereschi M, Pracucci G, Basile AM, Wolfe CD, et al. Sex differences in the clinical presentation, resource use, and 3-month outcome of acute stroke in Europe: data from a multicenter multinational hospital-based registry. Stroke. 2003 May;34(5):1114-9.

93. Vorster HH. Fibrinogen and women's health. Thromb Res. 1999 Aug 15;95(4):137-54.

94. Kannel WB, D'Agostino RB, Wilson PW, Belanger AJ, Gagnon DR. Diabetes, fibrinogen, and risk of cardiovascular disease: the Framingham experience. Am Heart J. 1990 Sep;120(3):672-6.

95. Hamsten A, de Faire U, Walldius G, Dahlen G, Szamosi A, Landou C, et al. Plasminogen activator inhibitor in plasma: risk factor for recurrent myocardial infarction. Lancet. 1987 Jul 4;2(8549):3-9.

96. Ossei-Gerning N, Wilson IJ, Grant PJ. Sex differences in coagulation and fibrinolysis in subjects with coronary artery disease. Thromb Haemost. 1998 Apr;79(4):736-40.

97. Haverkate F, Thompson SG, Duckert F. Haemostasis factors in angina pectoris; relation to gender, age and acute-phase reaction. Results of the ECAT Angina Pectoris Study Group. Thromb Haemost. 1995 Apr;73(4):561-7.

98. Kalaria VG, Zareba W, Moss AJ, Pancio G, Marder VJ, Morrissey JH, et al. Gender-related differences in thrombogenic factors predicting recurrent cardiac events in patients after acute myocardial infarction. The THROMBO Investigators. Am J Cardiol. 2000 Jun 15;85(12):1401-8.

99. Renner W, Brodmann M, Pabst E, Stanger O, Wascher TC, Pilger E. The V34L polymorphism of factor XIII and peripheral arterial disease. Int Angiol. 2002 Mar;21(1):53-7.

100. Catto AJ, Kohler HP, Bannan S, Stickland M, Carter A, Grant PJ. Factor XIII Val 34 Leu: a novel association with primary intracerebral hemorrhage. Stroke. 1998 Apr;29(4):813-6.

101. Corral J, Gonzalez-Conejero R, Iniesta JA, Rivera J, Martinez C, Vicente V. The FXIII Val34Leu polymorphism in venous and arterial thromboembolism. Haematologica. 2000 Mar;85(3):293-7.

102. Elbaz A, Poirier O, Canaple S, Chedru F, Cambien F, Amarenco P. The association between the Val34Leu polymorphism in the factor XIII gene and brain infarction. Blood. 2000 Jan 15;95(2):586-91.

103. Gemmati D, Serino ML, Ongaro A, Tognazzo S, Moratelli S, Resca R, et al. A common mutation in the gene for coagulation factor XIII-A (VAL34Leu): a risk factor for primary intracerebral hemorrhage is protective against atherothrombotic diseases. Am J Hematol. 2001 Jul;67(3):183-8.

104. Iniesta JA, Corral J, Gonzalez-Conejero R, Diaz Ortuno A, Martinez Navarro ML, Vicente V. Role of factor XIII Val 34 Leu polymorphism in patients with migraine. Cephalalgia. 2001 Oct;21(8):837-41.

105. Endler G, Funk M, Haering D, Lalouschek W, Lang W, Mirafzal M, et al. Is the factor XIII 34Val/Leu polymorphism a protective factor for cerebrovascular disease? Br J Haematol. 2003 Jan;120(2):310-4.

106. Kain K, Young J, Bavington J, Bamford J, Catto AJ. Coagulation factor XIII B subunit antigen, FXIIIVal34Leu genotype and ischaemic stroke in South Asians. Thromb Haemost. 2005 Feb;93(2):394-5.

107. Rubattu S, Di Angelantonio E, Nitsch D, Gigante B, Zanda B, Stanzione R, et al. Polymorphisms in prothrombotic genes and their impact on ischemic stroke in a Sardinian population. Thromb Haemost. 2005 Jun;93(6):1095-100.

108. Slowik A, Dziedzic T, Pera J, Figlewicz DA, Szczudlik A. Coagulation factor XIII VaI34Leu polymorphism in patients with small vessel disease or primary intracerebral hemorrhage. Cerebrovasc Dis. 2005;19(3):165-70.

109. Pruissen DM, Slooter AJ, Rosendaal FR, van der Graaf Y, Algra A. Coagulation factor XIII gene variation, oral contraceptives, and risk of ischemic stroke. Blood. 2008 Feb 1;111(3):1282-6.

110. Smith NL, Bis JC, Biagiotti S, Rice K, Lumley T, Kooperberg C, et al. Variation in 24 hemostatic genes and associations with non-fatal myocardial infarction and ischemic stroke. J Thromb Haemost. 2008 Jan;6(1):45-53.

111. Akar N, Donmez B, Deda G. FXIII gene Val34Leu polymorphism in Turkish children with cerebral infarct. J Child Neurol. 2007 Feb;22(2):222-4.

112. Casas JP, Hingorani AD, Bautista LE, Sharma P. Meta-analysis of genetic studies in ischemic stroke: thirty-two genes involving approximately 18,000 cases and 58,000 controls. Arch Neurol. 2004 Nov;61(11):1652-61.

113. Pruissen DM, Kappelle LJ, Rosendaal FR, Algra A. Genetic association studies in ischaemic stroke: replication failure and prospects. Cerebrovasc Dis. 2009;27(3):290-4.

114. Warner D, Mansfield M, Grant PJ. Coagulation factor XIII levels in UK Asian subjects with type 2 diabetes mellitus and coronary artery disease. Thromb Haemost. 2001 Oct;86(4):1117-8.

LIST OF ORIGINAL PUBLICATIONS RELATED TO THE DISSERTATION

Shemirani AH, Muszbek L. Rapid detection of the factor XIII Val34Leu (163 G-->T) polymorphism by real-time PCR using fluorescence resonance energy transfer detection and melting curve analysis. Clin Chem Lab Med. 2004; 42: 877-9.

Impact factor: 1.685

Shemirani AH, Haramura G, Bagoly Z, Muszbek L. The combined effect of fibrin formation and factor XIII A subunit Val34Leu polymorphism on the activation of factor XIII in whole plasma. BBA-Proteins Proteom 2006; 1764: 1420-3.

Impact factor: 3.311

Shemirani AH, Szomjak E, Csiki Z, Katona E, Bereczky Z, Muszbek L. Elevated factor XIII level and the risk of peripheral artery disease. Haematologica. 2008; 93: 1430-2.

Impact factor: 5.978

Muszbek L, Bereczky Z, Bagoly Z, **Shemirani AH**, Katona É. Factor XIII and atherothrombotic diseases. Semin Thromb Hemost. 2010; 36: 18-33.

Impact factor: 3.214

Shemirani AH, Pongrácz E, Antalfi B, Adány R, Muszbek L. Factor XIII A subunit Val34Leu polymorphism in patients suffering atherothrombotic ischemic stroke. Thromb Res. 2010; 126: 159-62.

Impact factor: 2.406

Total impact factors of original publications related to the dissertation: 16.594

ABSTRACTS OF PRESENTATIONS WITH RESULTS NOT INCLUDED IN THE PUBLICATIONS, BUT RELATED TO THE DISSERTATION

Shemirani AH, Antalfi B, Pongrácz E, Muszbek L. Factor XIII Val34Leu polymorphism in patients surviving or not surviving ischemic stroke. Pathophysiol Haemost Thromb 2007/2008; 36 Suppl 1: A13

Shemirani AH, Csiki Z, Szomják E, Katona É, Bereczky Z, Muszbek L. Factor XIII level and prevalence of FXIII-A Val34Leu polymorphism in patients with peripheral arterial disease and Raynaud's disease. Pathophysiol Haemost Thromb 2003; 33 (suppl2): 85a

LIST OF ORIGINAL PUBLICATIONS NOT CLOSELY RELATED TO THE DISSERTATION

Góth L, **Shemirani AH**, Kalmár T. A novel catalase mutation (a GA insertion) causes the Hungarian type of acatalasemia. Blood Cells Mol Dis. 2000; 26: 151-4.

Impact factor: 2.897

Tarnai I, Csordás M, Sükei E, **Shemirani AH**, Káplár M, Góth L. Effect of C111T polymorphism in exon 9 of the catalase gene on blood catalase activity in different types of diabetes mellitus. Free Radic Res. 2007; 41: 806-11.

Impact factor: 2.925

Bereczky Z, Balogh E, Katona É, Czuriga I, Kárpáti L, **Shemirani AH**, Édes I, Muszbek L. Decreased factor XIII levels in factor XIII A subunit Leu34 homozygous patients with coronary artery disease. Thromb Res. 2008; 121: 469-76.

Impact factor: 2.449

Shemirani AH, Szomják E, Balogh E, András C, Kovács D, Ács J, Csiki Z. Polymorphism of clotting factors in Hungarian patients with Raynaud's phenomenon. Blood Coagul Fibrin. 2010; accepted. DOI:10.1097/MBC.0b013e32834234fe.

Impact factor: 1.246

Total impact factors of original publications not closely related to the dissertation: 9.517

KEYWORDS

Atherothrombotic ischemic stroke

Factor XIII

Factor XIII activation

Factor XIII activity

Factor XIII antigen

FXIII-A Val34Leu polymorphism

Fibrinogen

Gender

Peripheral artery disease

Real time PCR

ACKNOWLEDGEMENTS

This dissertation would not have been possible without the guidance and the help of several individuals who in one way or another contributed and extended their valuable assistance in the preparation and completion of this study.

First and foremost, I wish to express my sincere gratitude to Prof. László Muszbek, whose guidance and support from the initial to the final level enabled me to complete my researches.

I offer my regards and blessings to all the colleagues at the Clinical Research Center and Clinical Biochemistry and Molecular Pathology Department who rendered their help during the completion of this work.

Last but not least I wish to avail myself of this opportunity, express a sense of gratitude and love to my beloved family for their support, strength, help, and for everything.