Association of plasminogen activator inhibitor-1, angiotensin converting enzyme and coagulation factor XIII genes polymorphisms with recurrent spontaneous abortion

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BY

OMNIA MAHMOUD ABD ALAZIZ
(B.Sc.)

Supervised by

PROF. DR. IMAN RIFAAT ELMAHGOUB
PROFESSOR OF CLINICAL PATHOLOGY,FACULTY OF MEDICINE
CAIRO UNIVERSITY

ASS. PROF. REHAM ABDELALEEM AFIFY
ASSISTANT PROFESSOR OF CLINICAL PATHOLOGY, FACULTY OF MEDICINE
CAIRO UNIVERSITY

ASS. PROF. WALED SAID ELSHERBINY
ASSISTANT PROFESSOR OF OBSTETRICS AND GYNECOLOGY, FACULTY OF MEDICINE
CAIRO UNIVERSITY

FACULTY OF MEDICINE
CAIRO UNIVERSITY
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Abstract

Background: Recurrent miscarriage (RM) is defined as three or more consecutive pregnancy failures. Polymorphisms of plasminogen activator inhibitor-1 (PAI-1), angiotensin converting enzyme (ACE) and factor XIII (FXIII) appear to be a cause of imbalance between coagulation and fibrinolysis which could promote the development of RM. **Aim:** The current retrospective case-control study aimed at detecting the association between PAI-1 4G/5G polymorphism, ACE intron 16 insertion/ deletion polymorphism and FXIII Val34Leu polymorphism and repeated spontanoeus abortions (RSA) in Egyptian women. **Subjects and Methods:** Genotyping of 50 RSA patients and 50 healthy controls by PCR amplification of the target gene followed by allele specific restriction enzyme digestion (RFLP technique). **Results:** Our results revealed that the frequencies of PAI-1 4G/5G polymorphism in the case group were 64% for the wild type versus 30% for the for the heterozygous type and 6% for the polymorphic homozygous type, while in the control group they were 76%, 22% and 2% respectively. The frequencies of ACE intron 16 insertion/ deletion polymorphism in the case group were 16% for the wild type versus 56% for the for the heterozygous type and 28% for the polymorphic homozygous type, while in the control group they were 22%, 36% and 42% respectively. The frequencies of FXIII Val34Leu polymorphism in the case group were 68.0% for the wild type versus 30% for the heterozygous type and 2% for the polymorphic homozygous type, while in the control group they were 100.0%, 0% and 0% respectively. **Conclusion:** FXIII Val34Leu polymorphism was found to be associated with increased risk of RSA, in this sample of Egyptian women.

Key words: Recurrent spontaneous abortion, polymorphism, plasminogen activator inhibitor-1, angiotensin converting enzyme, coagulation factor XIII.

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List of Abbreviations

ACE	**Angiotensin-converting enzyme**
aCL	**Anticardiolipin antibodies**
Anti-β2 GP-1	**Anti-β2 glycoprotein-1**
AP-1	**Activator protein 1**
APC	**Activated protein C**
APCR	**Activated Protein C Resistance**
aPL	**Antiphospholipid antibodies**
APS	**Antiphospholipid syndrome**
aPTT	**Activated partial thromboplastin time**
AT	**Antithrombin**
CS	**Chondroitin sulphate**
D	**Deletion**
D3	**Domain 3**
DS	**Dermatan sulphate**
EDTA	**Ethylene diamine tetra-acetic acid**
EMC	**Extracellular matrix**
EPCR	**Endothelial cell protein C receptor**
Factor XIIIa	**Activated FXIII**
FGG	**fibrinogen γ chain**
FDPs	**Fibrin degradation products**
FGR	**Fetal growth restriction**
FSF	**Fibrin stabilizing factor**
FVL	**factor V Leiden**
FXa	**Factor Xa**
GAG	**Glycosaminoglycans**
GPI	**Glycosyl phosphatidylinositol**
HA	**Hyaluronan**
HCII	**Heparin cofactor II**
His	**Histidine**
HS	**Heparan sulphate**
HSG	**Hysterosalpingography**
I	**Insertion**
IL-1	**Interleukin 1**
IL-6	**Interleukin 6**
LA	**Lupus anticoagulant**

LDLR	**Low denstity lipoprotein receptor**
Leu	**Leucine**
LMWH	**Low molecular weight heparin**
LRP	**Lipoprotein receptor related protein**
MCP-1	**Monocyte chemoattractant protein-1**
MTHFR	**Methylenetetrahydrofolate reductase**
NF-κB	**Nuclear factor-κB**
PA	**Plasminogen activator**
PAI-1	**plasminogen activator-inhibitor-1**
PAI-2	**plasminogen activator-inhibitor-2**
PAIs	**Plasminogen activator inhibitors**
PC	**Protein C**
PCR	**Polymerase chain reaction**
PN-1	**Protease nexin 1**
PROC	**Protein C gene**
PROS1	**Protein S gene**
PS	**Protein S**
RAS	**Rennin angiotensin system**
RFLP	**Restriction fragment length polymorphism**
RGD	**Arginine-glycine-aspartate**
RPL	**Recurrent pregnancy loss**
RSA	**Recurrent spontaneous abortion**
SMB	**Somatomedin-B**
SNP	**Single nucleotide polymorphism**
TAFI	**Thrombin-activatable fibrinolysis inhibitor**
TFPI	**Tissue factor pathway inhibitor**
TGF- β	**Transforming growth factor- β**
TNF-α	**Tumour necrosis factor-α**
tPA	**Tissue-type plasminogen activator**
UFH	**Unfractionated heparin**
uPA	**Urokinase –type plasminogen activator**
uPAR	**Urokinase –type plasminogen activator receptor**
Val	**Valine**
VEGF	**Vascular endothelial growth factor**
VTE	**Venous thromboembolism**
vWF	**von Willebrand factor**

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Introduction

Miscarriage is a common complication of pregnancy, occurring in 10 to 15% of pregnant women ***(Daya, 2003)***.Traditionally, recurrent miscarriage is defined as three or more consecutive pregnancy losses before the 20th week of gestation ***(Mtiraoui et al.,2007).*** It has been estimated that about 1- 2% of couples have 3 or more consecutive miscarriages and 5% of all couples have 2 miscarriages ***(Bricker and Farqusharson, 2002).***

The establishment of a successful pregnancy is a complex process, where the events taking place during early to mid gestation, from fertilization, implantation of blastocyst, differentiation of trophoblast to invasion of the endometrium by the trophoblast along with establishment of the feto–maternal interface, are brought about by careful regulation of the interplay of multiple factors which either infiltrate or are expressed in the uterine microenvironment ***(Hanna et al., 2006)***.

Various factors have been identified that influence miscarriage, including uterine anomaly, chromosomal abnormalities, endocrine dysfunction, thrombophilia, immune disorders, lifestyle factors and maternal infections ***(Regan et al., 1998).*** However, in up to 50% of patients who experience recurrent pregnancy loss, the underlying causes remain undetermined ***(Li et al., 2002)***.

The fibrin-stabilizing factor (FXIII) has been shown to play an important role in placentation during the first trimester of pregnancy**(Kappelmayer et al., 1994)**. Deficiency of FXIII with the resulting lack of fibrin stability and tendency towards hemorrhage is regarded as a risk factor for miscarriages ***(Anwar et al., 1999)***. The Val34Leu polymorphism in exon 2 of the FXIII-A

gene could have an antifibronolytic effect through the early cross-linking of fibrin fibers ***(Buchholz and Thaler, 2003)***.

On the other hand, for successful implantation, invasion of the cytotrophoblast to the proper depth of the uterus is crucial. It provides anchorage for the conceptus and promotes adaptation of uteroplacental circulation **(Feng et al., 2000)**. Urokinase plasminogen activator, its receptor, and plasminogen activator inhibitor 1 (PAI-1) are believed to control proteolysis and remodeling of maternal tissue during trophoblast *invasion* ***(Lockshin, 1999 ;Floridon et al., 2000)***. PAI-1 also has a vital role in hypofibrinolysis and thrombotic complications. The gene expression is modulated by a 4G/5G polymorphism in the promoter region, which is located 675 bp upstream from the start site of transcription ***(Balta et al., 2002).***

Angiotensin converting enzyme (ACE) plays a critical role in the renin–angiotensin system (RAS) and is involved in the conversion of angiotensin I to active angiotensin II, a potent vasopressor. ACE also has a physiological function in the fibrinolysis pathway as it regulates the concentrations of PAI-1, an important determinant in the control of the fibrinolytic process ***(Kim et al., 1997).*** The level of enzyme is related to polymorphism of ACE gene consisting of the insertion/ deletion (I/D) of a 287-bp fragment in intron 16 ***(Wiwanitkit, 2004).***

Aim of the work:

The aim of the study was to investigate whether presence of PAI-1 4G/5G polymorphism ,ACE intron 16 insertion/ deletion polymorphism and FXIII Val34Leu polymorphism increases the risk of recurrent spontaneous abortion (RSA) in Egyptian women.

Normal physiology of coagulation

Haemostasis is a complex process which prevents the spontaneous bleeding and controls traumatic bleeding. It depends upon normal blood vessels, normal platelet activity, adequate coagulation system and stability of the clot ***(Sirridge and Shannon, 1983).***

The coagulation cascade:

The coagulation cascade involves more than 20 proteases, cofactors and inhibitors. This cascade is a sequence of enzyme reactions, each being activated by the previous one, which once initiated proceeds to the final one. The essential advantage inherent in this process is the rapid biochemical amplification of a response. In such systems, proteins operate in pairs, one acting as enzyme, the other as substrate in turn ***(Kerr et al., 1975; Mullertz et al., 1984).***

Figure (1) illustrates the current model of blood coagulation which involves two distinct pathways; the primary pathway commonly known as the "extrinsic or the tissue factor pathway" and the "intrinsic or the contact activation pathway". These two pathways merge together with the formation of FXa, the serine protease in the prothrombinase complex responsible for the conversion of prothrombin to thrombin. Thrombin cleaves fibrinogen to fibrin, which polymerizes to form an insoluble fibrin clot. In addition, thrombin is the key activator of platelet aggregation at the site of injury ***(Davey and Luscher, 1967).***

Platelets form a plug that stops the hemorrhage and prevents further blood loss. Also, during the activation process a multitude of proteins is released at the site of injury initiating the process of tissue repair. These include von Willebrand factor (vWF) which plays an important role in forming a bridge between the activated platelets and the subendothelium. Platelet aggregation also promotes the clotting process, since activated platelets provide the phospholipid base required for the formation of the vitamin–K dependent coagulation enzyme complexes. The fibrin clot formed by the clotting cascade, complementarily strengthens the platelet plug ***(Girma et al., 1987).***

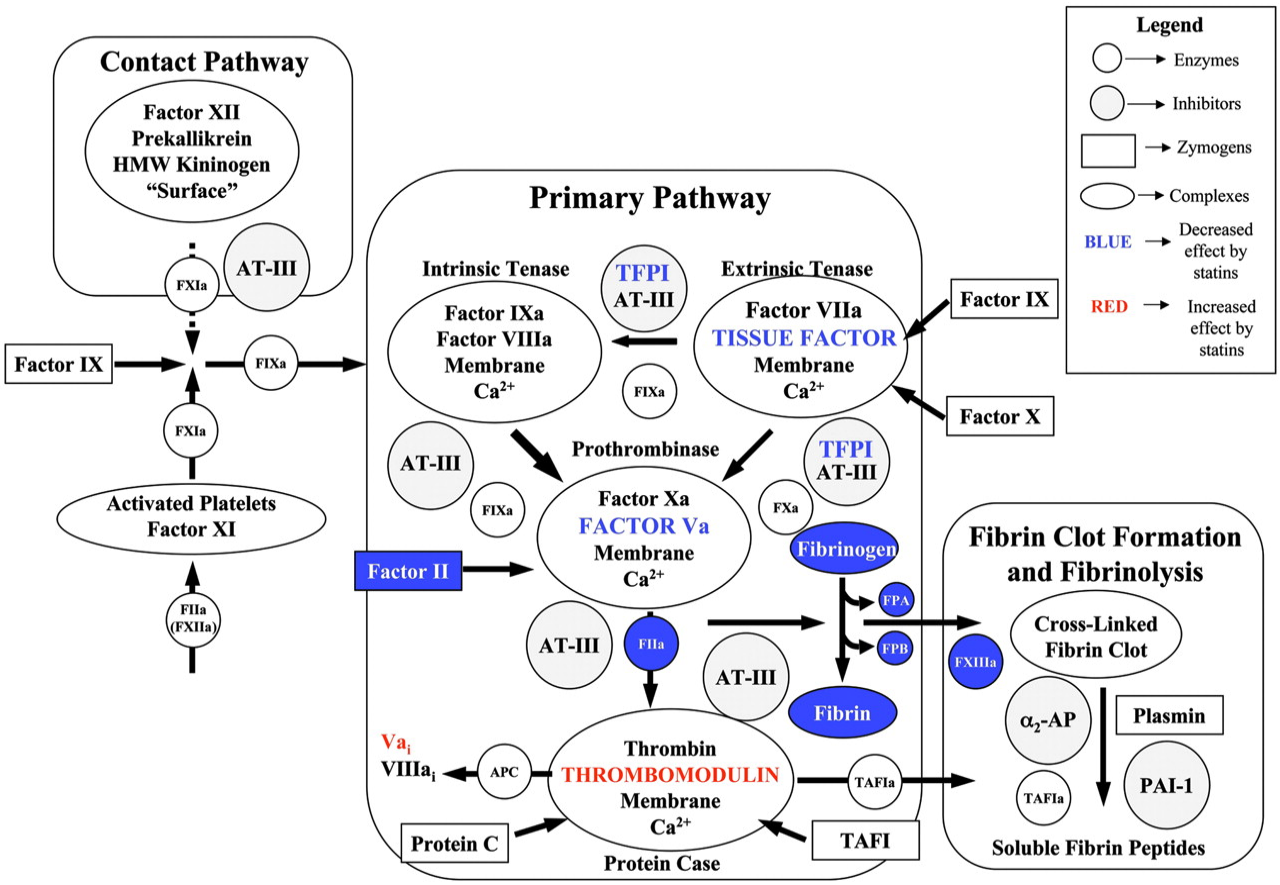

Figure (1): The current model of the blood coagulation cascade **(*Anetta et al., 2005)***

FXIII:

Factor XIII (FXIII) is the last enzyme in the clotting cascade. Its main function is to convert the loose fibrin polymer into a firm, highly organized, cross-linked structure with increased tensile strength, firmly anchored to the site of the wound and possessing an in-built resistance to fibrinolysis. In FXIII deficiency, standard clotting tests are normal, as the clotting end point is not affected by the absence of FXIII. It is the quality of the clot which is abnormal. Unless this is assessed, the diagnosis may be missed. Soon after its discovery by ***Robbins (1944),*** FXIII was aptly named; the fibrin stabilizing factor or FSF: Fibrin formed in the absence of FSF was unstable: it dissolved in weak acids, weak bases and 5M urea. Addition of a small amount of plasma to the system stabilized the fibrin, it was no longer soluble in these reagents and it was also more resistant to fibrinolysis. Solubility of clots in 1% monochloroacetic acid or in 5M urea still forms the basis of the standard laboratory screening test for inherited FXIII deficiency ***(Anwar and Miloszewski 1999).***

Initially it was believed that FSF combined with fibrin, acting as a kind of glue, sticking molecules of fibrin together ***(Lorand, 1950).*** Later it became obvious that FSF was an enzyme ***(Buluk et al., 1961)*** and was identified as a member of the transglutaminase family of enzymes ***(Aeschlimann and Paulsson, 1994).*** FXIII is the only transglutaminase found both intra-and extracellularly and the only one requiring thrombin as well as calcium for activation. Thus, in common with other clotting factors it exists as a pro-enzyme ***(Anwar and Miloszewski 1999).***

In contrast to other pro-enzyme clotting factors, it is the precursor of a transglutaminase and not of proteolytic enzyme. Activated FXIII (FXIIIa) cross-links α chains and γ chains of fibrin and covalently attaches α2 plasmin inhibitor to fibrin alpha-chains to strengthen fibrin mechanically and to protect it from fibrinolysis. In addition to being a clotting factor, FXIII is also an intracellular pro-enzyme present in platelets and monocytes/macrophages ***(Muszbek, 2000).***

In plasma, FXIII is expressed as a zymogen of the form α2β2. In the presence of thrombin and calcium, the α2 units is released and activated. By contrast, platelet FXIII is expressed as the zymogen α2 unit ***(Trumbo and Maurer, 2000).***

FXIII activation:

The process by which plasma FXIII is activated is quite complex. Thrombin plays a role in both the conversion of fibrinogen to fibrin and the formation of the fibrin-stabilizing enzyme, FXIIIa ***(Greenberg and Orthner, 1999).***

Plasma FXIII is a heterologous tetramer consisting of 2 A and 2 B subunits. The A subunits contain the enzyme's active site, and the B subunits serve a carrier function of the hydrophobic A subunit in the aqueous environment of human plasma. Activation of FXIII involves cleavage of the activation peptides from the A subunit, which then may

or may not dissociate from the complex. In a second step, calcium and fibrin induce the dissociation of the B subunits from A to expose the active site's thiol group as illustrated in Figure (2):

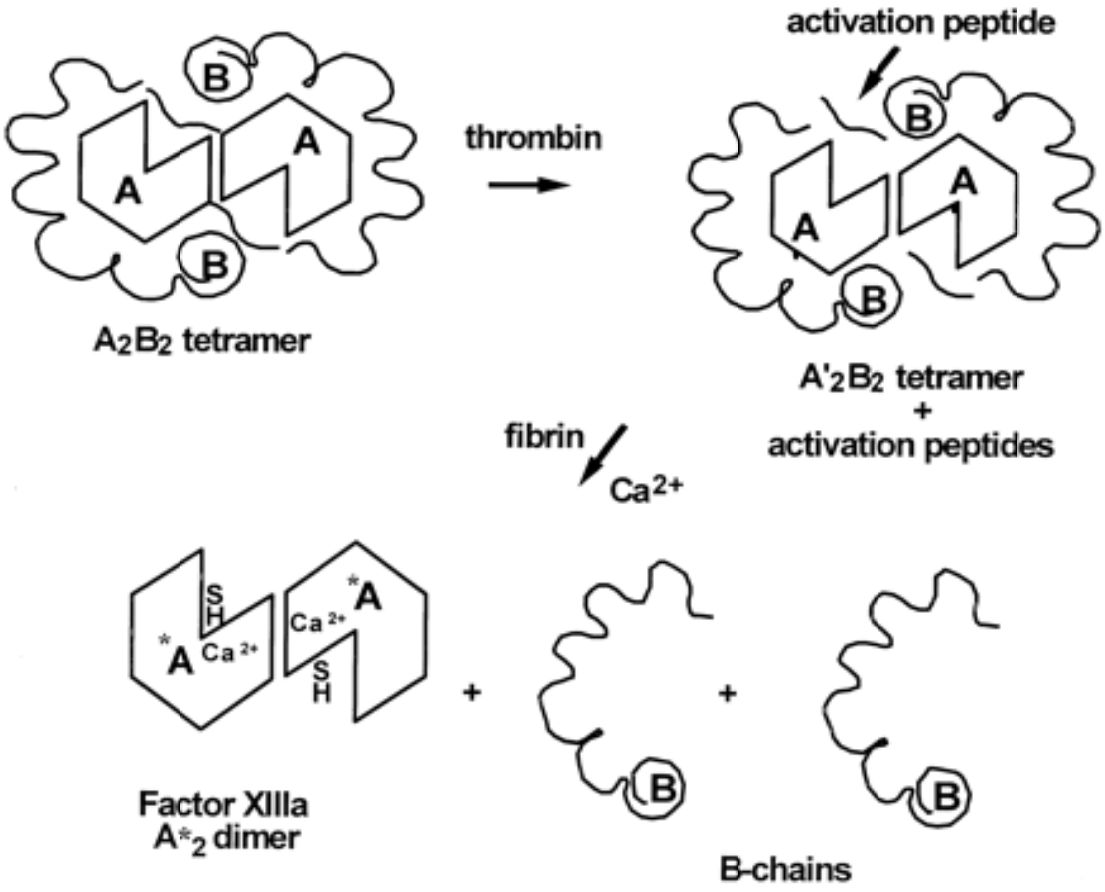

Figure (2): FXIII tetrameric structure and activation ***(Ariens et al., 2002).***

Thrombin cleavage of the A subunits is necessary to activate the plasma tetramer and dimeric platelet FXIII. Fibrin polymers are an important cofactor to generate FXIIIa ***(Hornyak and Shafer 1992).***