Coagulation Factor XIIIA (F13A1): novel perspectives in treatment and pharmacogenetics.

Gemmati Donato*[1], Vigliano Marco[1], Burini Francesco[1], Mari Rosella[1], Hossam Abd El Mohsein Hodeib[2], Parmeggiani Francesco[3], Serino M. Luisa[1].

[1]Ctr. Hemostasis & Thrombosis - Unit of Haematology, Dept. of Medical Sciences, University of Ferrara, Italy; [2] Unit of Haematology, Tanta University, Egypt; [3]Eye Clinic, Depart. of Biomedical and Specialty Surgical Sciences, University of Ferrara, Italy.

***Corresponding author: Donato Gemmati;**
Ctr. Hemostasis & Thrombosis - Unit of Haematology
Dpt. Medical Sciences,
University of Ferrara, I-44100, Ferrara ITALY
tel (+39) 0532.237291
fax (+39) 0532.209010
email: d.gemmati@unife.it

Summary

Factor XIII (FXIII) is a key molecule in the field of blood coagulation and in the last decades it has weakened attention within the field of angiogenesis and tissue repair. FXIII positively influences wound healing in several tissues by exerting multiple plasma and cellular functions. In the field of haemostasis, FXIII cross-links the neo formed fibrin fibers and supports platelet adhesion to the damaged sub-endothelium warranting a solid architecture. In addition, the pro-angiogenic functions of FXIII are directed by the interaction of vascular endothelial growth factor receptor 2 (VEGFR2) and the integrin $\alpha_V\beta_3$, on the cell membrane, favouring an important step in the formation of granulation tissue at the wound site for optimal tissue healing. Conversely, the same mechanisms could lead to undesired increased neovascularisation, for example in inflammatory bowel disease or in the retinal degenerative pathologies. The classical symptoms of FXIII deficiency span from intracranial haemorrhage to delay bleeding or the staying of chronic wounds in the skin including impaired mucosal healing. In this view, FXIII bridges primary haemostasis, coagulation and definite tissue healing. Another important recently discovered function ascribed to FXIII is its ability to limit bacterial spreading from the lesion by incorporating specific macromolecules addressed to cellular infiltration, favouring in turn cell migration and survival, as observed also in fibrin-heart cultures for stem cell recruitment. In the field of the novel prognostic biomarkers, the monitoring of the residual circulating FXIII level during acute myocardial infarction has been considered predictive of the post-myocardial infarction healing. Accordingly, adequate FXIII levels can drive and predict the prognosis of complex diseases and the outcome of the associated therapies or interventions. In addition, peculiar pharmacogenetics aspects of the FXIII gene are of extraordinary interest. The present review accounts for the recognized role of FXIII in the healing process and gives some examples on how to use it as prognostic biological/molecular marker or as potential tailored therapeutic molecule in complex diseases.

Key words: Coagulation Factor XIII (FXIII); wound-healing; tissue repair; inflammation; pharmacogenetics; cardiovascular diseases; degenerative disease; stem-cells.

Introduction

Coagulation factor XIII (FXIII) is a circulating transglutaminase that works in the final steps of blood coagulation cascade and it plays a pivotal role in maintaining the mechanical and functional integrity of fibrin clots. The zymogen circulates in plasma as a protransglutaminase consisting of two catalytic A-subunits with enzymatic function and two carrier B-subunits also responsible for preventing premature activation/degradation of the catalytic A-subunit [1, 2]. Circulating FXIII is associated to the γ'chain of fibrin through FXIIIB. The A-subunit contains the activation peptide consisting of 37 aminoacids (AP; R37-G38), the active domain, and the substrate-recognition regions. FXIII is present in plasma either as hetero tetramer bounded to the B-subunits (FXIIIA2B2) or alone (FXIIIA2) as intracellular homo-dimer [3]. FXIII is proteolytically activated (FXIIIa) by thrombin (FIIa), and by the releasing of the AP from the NH_2 terminus and in the presence of Ca^{2+}, the inhibitory B-subunits dissociate (Fig.1A). FXIII can alternatively be activated *via* the B2-subunits dissociation in the presence of high concentration of Ca^{2+} but in absence of any proteolytic cut (Fig.1B).

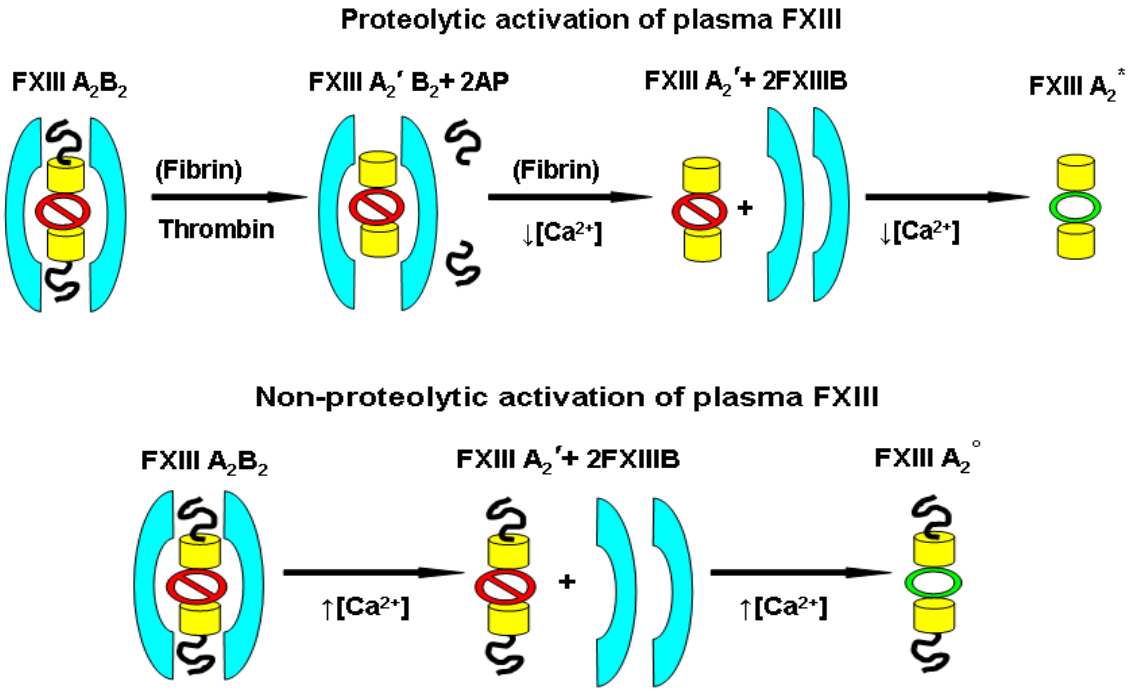

Figure 1 (A, B). Mechanisms of FXIII activation. A: Proteolytic activation of $FXIIIA_2B_2$ tetramer in presence of thrombin, fibrin and low concentration of Ca^{2+} ions; the activation peptide (AP) is cleaved from FXIIIA by thrombin. B: non-proteolytic activation of $FXIIIA_2B_2$ tetramer in presence of high concentration of Ca^{2+} ions, AP remains linked to FXIIIA. Cleaved activated FXIII ($FXIIIA_2$*); non-cleaved activated FXIII ($FXIIIA_2$°). FXIIIA (yellow), FXIIIB (light blue), inactive catalytic site (red), activated catalytic site (green).

This form resembles the intracellular isoform of the FXIIIA2 homo-dimer. All these steps originate the enzymatically active transglutaminase that catalyzes the covalent bonds between the γ-carboxyamine group of a glutamine residue and the ε-amino group of a lysine residue. This action in turn creates covalent cross-links between fibrin γ-dimers and among α-chains of fibrin monomers/polymers (Fig. 2) and covalently binds antifibrinolytic proteins to the fibrin network to improve chemical and physical properties [4].

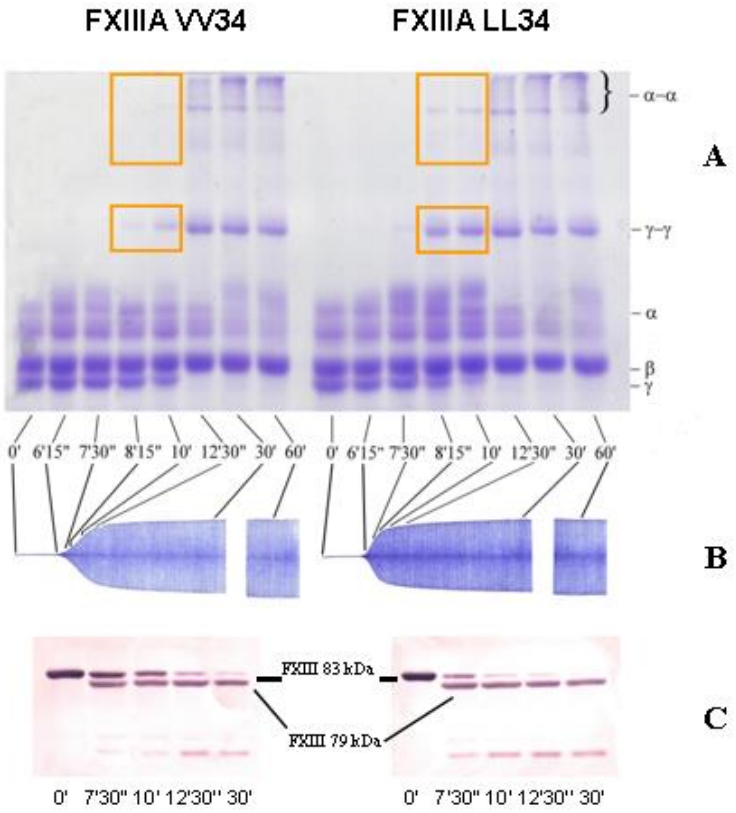

Figure 2. Fibrin cross-linking induced by activated FXIII (Thrombin 0.5U/ml, Ca^{2+} 8mM, Fibrinogen 1U/ml, FXIII 1U/ml at 37°C). A: SDS-PAGE under denaturing conditions of fibrin monomers (α, β, γ). B: Thrombelastogram (TEG) under the same experimental conditions as in A. Lag-phase was 7'30'' in VV34- and shorter (6'15'') in LL34-sample. C: Western-blotting analysis of activation of FXIII (1U/ml) under the same experimental conditions as in A. At 10 minutes of incubation FXIIIA LL34 was completely activated by thrombin (>90% of the cleaved 79kD form), compared with FXIIIA2 VV34 which retains large part of non-cleaved form (>40% of the full-length 83kD form). The full time-course was 60 min. The whole experimental procedures (A, B, C) were carried out at the same time. Left panel: FXIIIA VV34-homozygote, right panel: FXIIIA LL34-homozygote. It is to note the precocious appearance of the γ-γ dimers and α-polymers in the LL34 sample (red squares).

FXIIIA is mainly expressed by the cell lineages of megakaryocytes/platelets, and by monocytes/macrophages, as well as by their early precursors [5]. Platelets express about 3% of the total FXIIIA [6]. In addition, placenta, chondrocytes and osteoblasts also express FXIIIA [7]. The cellular origin of FXIII is quite controversial. Some studies ascribed to platelets or to monocytes/macrophages the main source [7-9], though during liver disease a general and contextual decrease of both A2 and B2

subunits has been observed, probably as the decreased protective effect role of the B-subunit of hepatic origin [10]. Finally, during bone marrow failure Kupffer cells, connective tissue histiocytes and hepatocytes could be extra-hematopoietic sources of FXIIIA [11]. FXIIIB subunit is released into the plasma by hepatocytes in a dimeric form (B2). FXIIIB2 stabilizes the FXIIIA2 increasing in turn its plasma half-life in the tetrameric form (A2B2) [12]. This action is probably obtained by the covering of the proteolytic cleavage sites (elastase, cathepsin, MMP-9), it might also prevent the spontaneous activation of FXIIIA in the circulation [13].

The wide gamma of FXIII substrates reflects the diverse effective roles of the molecule in different fields spanning from hemostasis/thrombosis to fibrinolysis, healing or angiogenesis [4]. Accordingly, we can have several different proteins to be considered as potential cross-linking substrates, and some of these are represented in table 1.

Table 1. Factor XIIIA substrates.

Protein	Substrate
Coagulation Factors	
	Fibrinogen α-chain
	Fibrinogen γ-chain
	Factor V
Fibrinolytic Proteins	
	α_2-Plasmin inhibitor (α_2-antiplasmin)
	Lipoprotein A
	Plasminogen
	Procarboxypeptidase B/U (Thrombin-activable Fibrinolysis Inhibitor; TAFI)
Adesive/Matrix Proteins	
	Collagen
	Thrombospondin
	Vitronectin
	Fibronectin
	Osteopontin
Cytoskeletal Proteins	
	Vinculin
	Actin
	Myosin
Others	
	AT_1 Receptor
	Semenogelins I and II
	Protein Synthesis Initiation Factor 5A
	α_2-Macroglobulin

The classical substrates for FXIII are the fibrin polymers and the fibrinogen, responsible for the plug formation in the final steps of blood coagulation with haemostatic purpose. This phase does not exhaust with clot formation, additional molecules are trapped in the fibrin network to improve its mechanical and elastic properties. Antifibrinolytic proteins such as α2-antiplasmin, α2-macroglobulin, and thrombin activable fibrinolysis inhibitor (TAFI) are responsible for the protection of the neo-formed fibrin network by earlier and premature degradation by endogenous fibrinolytic possesses [4, 14]. At the same time, bridging the end of the haemostatic functions (i.e. fibrin cross-link), including fibrinolysis (e.g. α2-AP cross-link) and angiogenesis (e.g. VEGFR2, $\alpha_V\beta_3$ integrin, TSP1 inhibition), FXIII promotes the healing phase responsible for the definitive tissue repair. Other macromolecules of the extracellular matrix (ECM), such as fibronectin, vitronectin, collagen, laminin, factor V are also cross-linked to fibrin, straddling coagulation-fibrinolysis and wound healing (Fig. 3). All these actions firstly optimize and synchronize the recruitment of macrophages and neutrophils and other leukocytes through integrin signalling pathways, and afterward the fibroblasts spreading and proliferation.

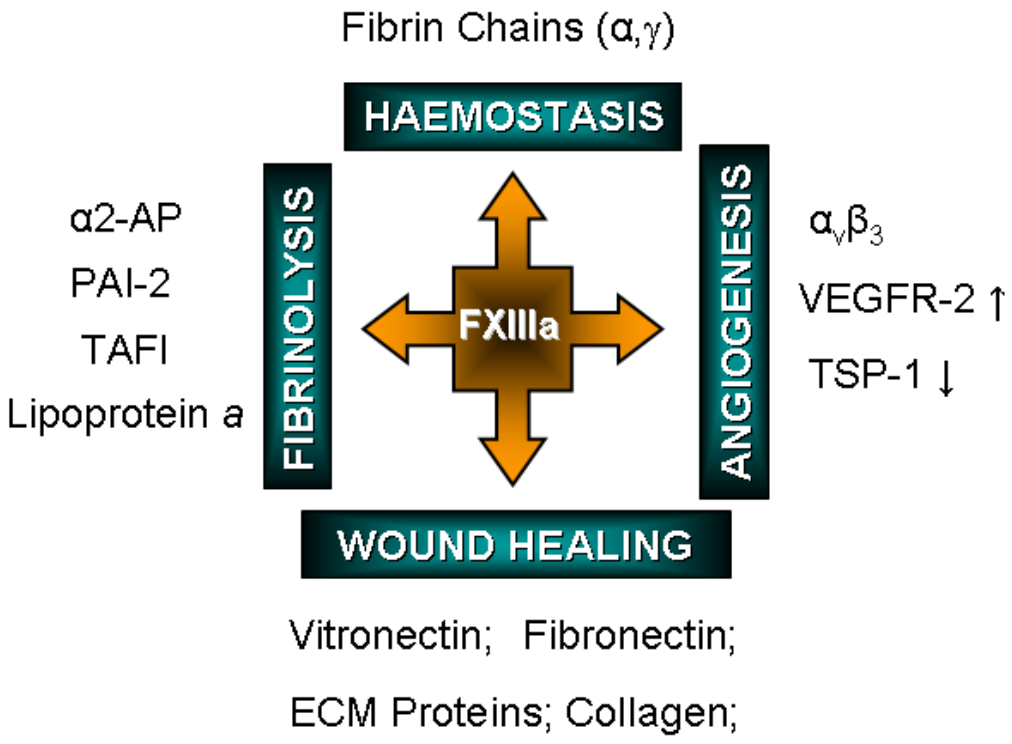

Figure 3. Schematic representation of the multitask role of activated FXIIIa and the relatives different substrates.

By means of these functions, FXIII enhances clot stability and also the epithelial and mucosal barrier functions, acting on platelet adhesion, reducing oedema formation, limitating bacterial intrusion, and favouring the infiltration and survival of inflammatory cells and fibroblasts. Finally, by promoting the angiogenic signalling, the global wound-healing capacity increases, resulting indeed very often affected in FXIII-deficient subjects.

Although FXIII gene is highly polymorphic (Table 2), its biological functions are mainly influenced by a unique specific single nucleotide polymorphism (SNP), the common G-to-T transversion at nucleotide 185 (G185T) in codon 34 (FXIIIA V34L). It is considered the main functional SNP of FXIIIA-subunit among the individuals of Caucasian descent [15]. It is located very close to the thrombin cleavage site, and affects the activation of FXIII *via* thrombin, the cross-linking activity and the tridimensional organization of the fibrin structure [16, 17]. Both VL- and LL34 genotypes are related to significant changes in the activation rate of FXIII that is increased in the LL-homozygotes and exhibits an intermediate effect in the heterozygous carriers [16]. Evident experimental *in vitro* proofs are provided by the thrombin activation of FXIII and by the cross-linking of fibrin evaluated in the two different homozygous genotypes, showing an increased activation rate in LL-homozygotes as demonstrated in western blotting analysis (Fig. 2 C) and in SDS-PAGE (Fig. 2A) respectively. Moreover, thrombelastography (TEG) analysis accounts for the earlier cross-linking activity (shorter lag phase) and the increased polymerization rate (slope) on fibrin observed in LL34-homozygote (Fig. 2B).

On these bases, it becomes clear that FXIII is a multifunctional protein playing key roles in both physiological and pathological processes [2, 18, 19]. The aim of this review is therefore to provide an overview of the peculiar and novel FXIII functions and to support its possible therapeutic use in selected patients, accordingly to their potential to clot/heal assessed by FXIII level monitoring together with pharmacogenetics information.

Table 2. Factor XIIIA polymorphisms and allele frequency in different populations.

Nucleotide Variation	**aminoacid variation**	**Exon**	**Allele Frequency** (ancestral/polymorphic)			
			Caucasians	**Africans**	**Japanese**	**Chinese**
F13A1						
c.G103T	p.V34L	2	0.767/0.233	0.883/0.117	1	1
c.A614T	p.Y204F	5	0.983/0.017	1	1	1
c.C1694T	p.P564L	12	0.758/0.242	86.4/13.6	0.705/0.295	0.733/0.267
c.T1766A	p.L588Q	13	0.975/0.025	1	1	1
c.G1951A	p.V650I	14	0.95/0.050	0.942/0.058	0.909/0.091	0.911/0.089
c.G1954C	p.E651Q	14	0.775/0.225	0.742/0.258	0.909/0.091	0.911/0.089

Codon position was according to Ichinose et al. ref. [109]; Allele frequency was according with International HapMap project (www.hapmap.org);
Caucasians: Utah resident with ancestry from Northern and Western Europe; Africans: Nigeria; Japanese: Tokyo; Chinese: Beijing.
Modified from Muszbek et al. ref. [18].

FXIII deficiency, classical symptoms and FXIII therapy

According to the *data* presented in literature, the frequency of FXIII deficiency is estimated to be about one in two million individuals and this yields to no more than a few thousands of patients worldwide [20-22]. The classical symptoms of FXIII deficiency include a delayed general bleeding tendency, umbilical cord bleeding after birth, muscle haemorrhage, mucosal bleeding, postoperative bleeding, severe intracranial haemorrhage, impaired wound healing and pregnancy loss often observed in the congenital FXIII deficient patients with the rare autosomic recessive trait.

Congenital FXIII deficiency is an autosomic recessive defect affecting the gene encoding FXIIIA subunit (F13A1), or less frequently, the gene encoding FXIIIB subunit (F13B). This latter may lead to reduced stability of freely circulating FXIIIA2 homo-dimer, due to the lack of the protective B-subunit [20, 23]. A significant number of patients experiences the acquired FXIII deficiency, often caused by autoimmune conditions and although not so easy to detect and discriminate by conventional laboratory techniques, it is not to be considered a rare clinical situation [24]. The generation of antibodies directed against FXIII, may affect all the steps directly or indirectly involved in the final FXIII activity: the activation rate of the molecule, the specific activity, the binding to fibrin or to other substrates, and even its circulating half-life [23, 25]. As regards FXIII deficient females, most of them experience recurrent pregnancy loss, though it has been reported successfully pregnancies also in females with low circulating FXIII levels [26-28]. The threshold level to have clinical manifestations is below 3-5% of the normal distribution. Additionally, low FXIII levels, can be caused by impaired synthesis, as can be observed following chemotherapy [8, 29] and chronic liver failure [10, 30], as well as in recurrent bleeding, inflammation, disseminated intravascular coagulation, burn wounds, sepsis, after open heart surgery, in inflammatory bowel disease [31-36], and also, as recently definitively demonstrated, in the acute phases of myocardial infarction [37].

FXIII concentrate (human or recombinant) is recommended as prophylactic treatment for patients with congenital FXIII deficiency [38, 39]. As the recombinant molecule is concerned, a recombinant analogue of FXIIIA subunit has been produced in yeast cells by Novo Nordisk [40]. The new

recombinant FXIII (rFXIII) was originally developed by Zymo Genetics and later it was acquired by the Novo Nordisk Company (Copenhagen, Denmark). The molecule was expressed in yeast (*Saccharomyces cerevisiae*) without the inclusion of human molecules [41]. The rFXIII once in plasma, associates with the free endogenous FXIIIB subunit to form the stable FXIII hetero-tetramer (FXIIIA2B2). In a recent study, patients with congenital FXIIIA subunit deficiency were infused with the recombinant molecule (Tretten®), which was effective in preventing bleeding in 90% of them when given monthly, according to the FDA [42]. Tretten® was approved by the FDA in 2013 from a clinical study, demonstrating safety and efficacy of the recombinant molecule. Trial phase 3 showed that preventive treatment with 35 IU/kg/month significantly decreased the number of at risk episodes needing treatment [40]. Two additional FXIII concentrates characterized by intermediate-purity have been tested in the USA and are actually available for clinical purposes in worldwide. They are virus-inactivated FXIII concentrates obtained from human plasma or placenta. Corifact® (CSL Behring) is the human FXIII concentrate approved in USA. Fibrogammin P® (CLS Behring) is the plasma-derived concentrate distributed in Europe, South America, Japan and South Africa. Finally, the FXIII concentrate of Bio Products Laboratory (Elstree, Hertfordshire, UK) is available on request.

FXIII role in complex diseases

Delayed wound healing in patients with low FXIII levels is known from long time and together with chronic venous lesions, account for a significant portion of patients in which it has a role. Several papers have been published in which FXIII levels, activity and peculiar genotypes have been demonstrated to have significant effects on the risk of establishment, progression and prognosis of chronic venous lesions [43-47].

Several theories and etiopathogenetic mechanisms have been proposed to explain dermal abnormalities in chronic venous lesions, accounting a complex interplay that involves venous hypertension, inflammation, cytokines and matrix MMPs activation, resulting in altered cellular functions and delayed wound healing [48] in which FXIII together with other different actors is one of

the leader molecules directly or indirectly involved in contrasting detrimental/degenerative and in favouring pro-healing/reparative processes [49]. Other molecules, belonging to the iron metallobiology, have long been suspected as causal agent in venous leg ulcer pathophysiology in close relation with FXIII molecule [50]. The mechanism by which they deregulated iron cycle is mediated by the generation of free oxygen radicals (ROS) and/or activation of MMPs or else down-regulation of tissue inhibitors of MMPs. FXIII counteracts the detrimental action of ROS and MMPs improving both physical and mechanical strength in the ECM protein network against unrestrained proteolytic action [49-52]. An *in vitro* direct evidence of such a suggested mechanism, it has been provided by the positive effect of FXIII in term of cell viability in fibroblast cell culture [51]. Physiological FXIII concentrations significantly counteracted the proteolytic action of MMPs and gave to cells normal growth rate and regeneration in a dose dependent manner [51]. Not only the levels and activity of endogenous FXIII have been investigated in chronic lesions, but also exogenous applications on the lesion of commercial FXIII concentrated and a favouring healing function has been demonstrated *in vivo* and *in vitro* [51, 53-57].

On the basis of these information, pharmacogenetics prevention programs have been proposed to help and influence the clinical practise in terms of predicting lesion onset or progression, stratifying patients according to their potential to heal. For the first time in surgery they have been considered algorithms influencing clinical procedures, taking into account the different genetic background and the clinical phenotype of patients [44, 47, 58].

Regarding cardiovascular diseases, and in particular the post-myocardial infarction healing, it is to take into account that, apart plasma FXIII tetramer (FXIIIA2B2), FXIIIA2 homo-dimer is present in platelets, monocytes and macrophages, all cells actively involved in infarct healing [2, 3, 59-61]. One of the first extraordinary evidences that directly demonstrated the essential role of FXIIIA in the stability of infarct scar has been obtained by Nahrendorf (Mention Year) from an experimental animal model [62]. In this study, 100% of mice with genetically reduced FXIIIA levels died within five days after induced myocardial infarction (MI). Left ventricular rupture was the cause of death, and no mice died due to severe bleeding or internal haemorrhage. Accordingly, intravenous FXIII treatment gave back a survival rate comparable to that of wild-type mice, though the cardiac MRI revealed an anomalous left ventricular

remodelling responsible for poor heart performances. The role of FXIII in supporting the post-MI healing was further confirmed and demonstrated by additional studies suggesting and supporting the use of FXIII as supplementary therapy to avoid anomalous left ventricular remodelling and loss of heart functions [63-65]. In addition, FXIII-based advanced treatments have been recently proposed to counteract the negative post-MI effects suggesting even intra-myocardial injection of FXIII-modifiable biomaterial [66, 67]. Finally, recent papers dealt with the role of platelet rich plasma (PRP) in MI healing. Intra-myocardial injections of autologous PRP have been successfully utilized during MI to accelerate and optimize local healing and contrast ROS-generation in the ischemic/reperfused heart [68-70]. As mentioned before, platelets contain FXIIIA together with a wide range of growth factors (GFs) and citokines (CKs), and after endogenous activation they release a huge and wide panel of pro-healing molecules at the injury site. By organizing a robust and elastic tri-dimensional fibrin network and influencing the ECM components (either cells and proteins), FXIII becomes promoter of additional important tasks such as adult staminal cells recruitment, neo-angiogenesis, collagen deposition leading overall to myocardial healing [18, 19, 71, 72]. Since it is hard to think about a deficiency of GFs/CKs in patients, we could rather hypothesize a “deficit” in the cell recruitment/anchoring (i.e. PLTs and white cells) by the tri-dimensional fibrin network, responsible in turn for a poor local GFs/CKs release and poor healing. FXIII finely tunes and modulates the fibrin-network for optimal storage and balanced release of GFs/CKs at the heart injury site. In this view, it could be considered the director of the forming of a sort of durable bio-patch with paracrine effect (GFs/cyto/chemiokines) or the driver of a bio-dispenser of pro-healing molecules, directly to the local injury site. A so complex organized fibrin network is the essential requisite to immediately counteract anomalous infarct healing and its extreme consequences such as heart rupture or development of severe heart failure (HF). Finally, such organized fibrin architecture is more attractive and efficacious also for endogenous/exogenous (stem) cells integration. Accordingly, it has been recently demonstrated that during MI and HF there is an increase of peripheral blood CD34+ stem cells and circulating endothelial progenitor cells (EPCs), reflecting a response to the endothelial damage [73]. How to improve cell mobilization/number and help their integration/proliferation still remains an exciting challenge. Generally, stem cell-based therapies are not

so efficacious because transplanted cells do not completely survive in the cardiac tissue. Conversely, it has been demonstrated that cross-linked fibrin-heart cultures are able to recruit increased number of stem cells *in vitro* with the possibility to reach therapeutic quantities of these cells [72, 74, 75]. Therefore, the warranty of optimal FXIII circulating levels, will ensure its efficacious action at the wound site. Thus, this could avoid invasive approaches, such as intra-myocardial injections, or eliminate alternative complex strategies, such as the construction of pre-constituted or self-assembling bio-scaffold, merely by improving the heart ability to self-heal. Two studies in the late 1970s investigated FXIII fluctuations after ischemic events, speculating a possible repair mechanism of the heart lesion [76, 77]. More recently, FXIII fluctuations in the acute venous or arterial accidents have been investigated with diagnostic/prognostic approach [78-82]. We recently demonstrated that during the first days after MI FXIIIA undergoes to an acute and transient fall in circulating levels and this occurs in the majority of patients [37]. Interestingly, patients undergoing excessive FXIII consuming at the time of MI were more prone to die or to develop HF earlier. Taken together, these data strongly support the idea to consider FXIII a prognostic biomarker and that appropriate FXIIIA circulating levels, or even better its availability at the injury site, are mandatory for optimal myocardial healing being the earliest phases extremely crucial also in the view of improved local stem cells recruitment.

Regarding inflammatory bowel diseases (IBD), they include disparate chronic intestinal diseases and among these, ulcerative colitis (UC) and Crohn's disease (CD) are the two most investigated due to their high prevalence [83, 84]. One common characteristic of these chronic conditions is the fact that FXIII is reduced and inversely relates with disease activity [85-87]. An increased FXIII consumption, due to the continuous attempts to regenerate tissue, together with the "activation/stopping" of blood coagulation in the inflamed tissue, might be the cause. Different studies investigated the efficacy of FXIII in counteracting IBD but final results are albeit controversial [88-92]. The physio-pathological bases lay on the fact that FXIII stabilizes the mucosal barriers, increases platelet adhesion containing a huge amount of growth factors and cytokines, and also contrasts establishment of oedema and bacterial invasion, helping regrowth of the intestinal epithelium and wound healing [2, 18, 19, 93]. The recently demonstrated protective role of FXIII in early innate immune defence [94, 2, 95], is in line with the

reduced ability of these patients to contrast bacterial infiltration at the site of mucosal lesion, according to their reduced cross-linking activity [96]. Epithelial restitution is of extraordinary importance in IBD patients. It's to note that all the main weaknesses in IBD etiopathogenesis are theoretically competence of and/or solvable by FXIII molecule: mucosal ulceration, angiogenesis, excessive bacterial burden in the mucosa, intense inflammation, whose long-standing give-up to restrained angiogenesis. Angiogenesis should be indeed considered as a dual and opposite matter; on one hand absolutely useful in regeneration of damaged tissues, but on the other hand responsible for leaky capillaries and weakening of endothelial barrier, favouring, oedema, exudation, inflammation, and tissue lesion, when acting under low FXIII effect and becoming unrestrained.

Novel and unconventional drug utilizations of FXIII

FXIII as a stand-alone treatment in bleeding

After the above supplied selected examples on the role of FXIII in complex diseases, in which similar and comparable mechanisms may lead to the loss of equilibrium in tissue regeneration and healing in different disease settings, it is now suggested an interesting novel approach to the treatment of bleeding, with a particular emphasis toward haemophilias [97, 98]. In this contest, FXIII concentrated has been suggested as unique drug in the treatment of haemophilic patients or in combination with other recombinant molecules [99]. Fibrinogen and FXIII are strongly related in haemostasis, with fibrinogen playing an important role also in platelet aggregation (primary hemostasis) and in the establishment of the final fibrin network (secondary hemostasis). The rationale is that during bleeding, regardless its aetiology, treatment must be rapid and specific towards the impaired pathway involved. Fibrinogen-to-fibrin conversion and the associated FXIII-mediated cross-linking processes are key events in providing effective haemostasis and stable clot. The crucial step to take into consideration is to have sufficient thrombin generation to sustain clot formation. Among the several actors involved in efficient haemostasis FXIII thanks to fibrin monomer cross-linking emerges as key molecule. It has been recently described a discordant fibrin formation in haemophilia resulting in clots less resistant to endogenous fibrinolysis (43% lower mass) [100]. Moreover, it has been suggested that increased fibrin monomer

levels are suggestive of high intraoperative bleeding. This should be due to an unbalanced thrombin-mediated fibrin/FXIIIa ratio. Very interesting is the observation that a single dose of FXIII (30U/kg) significantly reduces intra- and/or peri-operative bleeding. Finally, the joined effect of recombinant FVII (rFVIIa, NovoNordisk) and rFXIIIA was evaluated in clot lyses assays confirming that the two molecules contribute to clot formation and stability by complementary and different mechanisms eligible for a combined treatment [99, 101]. The effects of FXIII on clot stability and physical clot structure have been effective also at low concentrations of FVIII, indicating that FXIII is useful in severe defects [98]. So, FXIII may be considered as a stand-alone treatment for patients with mild to moderate haemophilia, with significant bleeding symptoms or even it may be useful in factor-sparing prophylaxis regimes [97]. It could be speculated that rather than specific targeted coagulation factors prophylaxis, FXIII alone might be sufficient to prevent bleeding in several and different contexts regardless the responsible deficient factor or the origin of bleeding, by ensuring the formation of structurally normal and stable clots. Finally, FXIII could also have effect in bypassing the treatment resistance and inefficacy in patients with inhibitory antibodies [98]. FXIII together with fibrinogen could be therefore proposed as a novel perspective in the treatment of coagulopathic bleeding.

FXIII as a Heart-healing

Myocardial infarction (MI) is the most frequent cause of heart failure (HF). In 2008 in the United States, about six-million people suffered from HF, and about 300.000 of them died. Reduced acute infarct mortality, due to efficient acute care (PTCA and thrombolysis), and insufficient options to treat infarct survivors chronically, have contributed to increased HF prevalence and hospitalization [102, 103]. Long term mortality due to HF is the crucial unsolved problem in MI survivors. Available standard treatments (ACE; β-blockers) help but do not eradicate the loss of heart performances after MI. New therapeutic strategies are needed and among them several promising molecules and strategies have been identified to potentially regenerate functioning myocardial cells. The recent development of imaging techniques, aimed at investigating heart healing, accounts for the monitoring of the so-called "*healing-biomarkers*". Among these, chemokines, growth factors, adhesion molecule, MMPs and

transglutaminases (i.e. FXIII), have been recognized with different timing and role in the complex post-MI healing phases (see detailed references in ref. 104). Briefly, acute infarction is responsible for the formation of a lesion in the wall of the culprit heart and this, together with the effects of the chronic high pressure and the heart beating, is considered the main cause of the anomalous remodelling of the left ventricle when not properly healed [105]. Contextually, an astonishing intrinsic wound-healing starts during the first one-two weeks after MI. This is a potential time to perform a therapeutic approach to prevent the anomalous wall remodelling and in turn HF. Loss of heart symmetry and affected geometry lead to a myocardium not properly working and destined to fail. This affects heart performance and in the long period inevitably causes HF. No specific and effective drugs till now there exist. During this period, the infarct lesion is highly active biologically and an early provisional fibrin mesh is formed. During these extensive changes of tissue architecture, the vulnerable wound is exposed to the mechanical stress of heartbeat and pressure, and the fibrin mesh growing could be hampered. Together with the damaged tri-dimensional fibrin meshwork, the whole healing process suffers from the continuous "*stop and go*". So, the heart wall can undergo toward deleterious changes in geometry and associated functions. In the short term, poor healing can lead to infarct expansion, left ventricular dilatation, wall rupture and death. In the long term, filling pressure, wall stress, and left ventricular volume can increase and propagate adverse remodelling, leading to HF and a poor prognosis. Among the physiological potential factors that can help the heart to heal, transglutaminases (i.e. FXIII) have been considered pro-survival factors and treatment procedures have been suggested so that FXIII has been called the "*cement of the heart*" [62-65]. Our group recently recognized a drastic fall in the circulating FXIIIA levels during the first days of MI, and this was genetically pre-determined [37, 81]. In addition, we recognized a FXIIIA threshold, and below that the prognosis was poor and HF or wall rupture had higher chance to occur during early follow up [37]. FXIIIA level significantly correlated with the worst prognosis independently from troponin (TnT) or CPK levels, then ascribing to FXIIIA the role of a novel independent prognostic marker in MI. In Figure 4, the levels of FXIIIA are compared with those of CKMB and TnT, FXIIIA peaked (lowest levels) at day 5, though it was informative starting from day 2 [37].

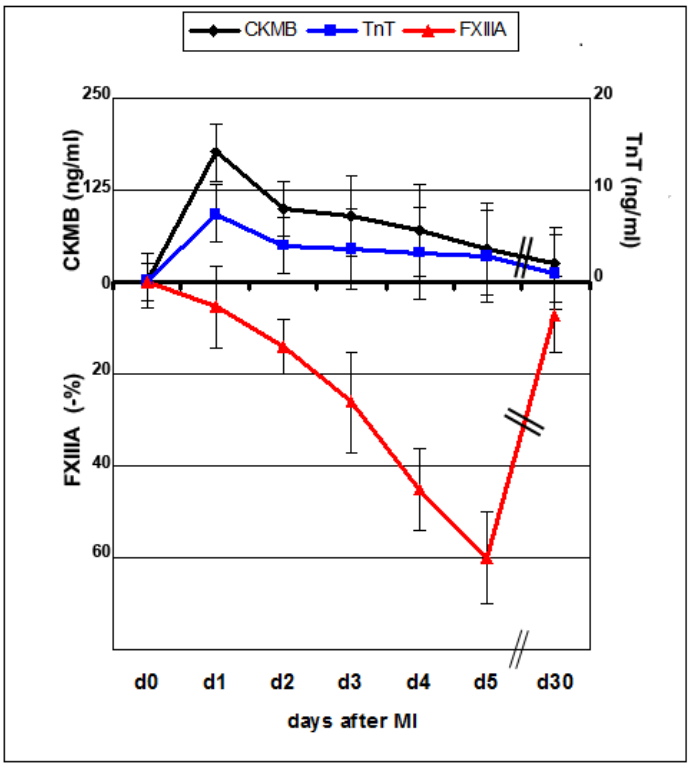

Figure 4. Kinetic comparison of the conventional myocardial infarction markers (CKMB mass and TnT) and FXIIIA assessed every 24h during the first days (d0-d5) after infarction; d30 indicates baseline levels assessed 30 days after the acute phase. It is to note the complete recover of FXIIIA concentration at day 30. (Modified from Gemmati et al. ref. [37]).

For these reasons, FXIIIA monitoring is strongly suggested in acute MI possibly together with the conventional ischemic biomarkers, and when below a certain threshold a FXIIIA restablishing treatment could be necessary. This potential at risk procedure should anyway be considered safe and viable only after definite clinical validations aimed at assessing the safety of the infusion of a pro-coagulant factor in MI patients. On the other hand, the monitoring of FXIIIA should select in advance those really in need of treatment, and large part of MI patients immediately start anti-aggregants and anti-coagulant drugs, so they could be considered protected having a poor haemostatic balance. Finally, supra-normal recombinant FXIIIA levels in healthy individuals have been considered not at risk situation [106, 107].

Novel Pharmacogenetics aspects of FXIIIA gene

The gene coding for human FXIIIA (F13A1) lies on chromosome 6p24–25 and spans over 160 kb [108]. The protein F13A1 contains 15 exons and 14 introns [109]. The mRNA is a transcript of about 3.9 kb. The AP is located in the exon II, being the exon I is completely in the non-coding region. FXIIIA

384 gene is highly polymorphic and wide differences in racial distribution have been demonstrated [110].
385 Several gene variants have been described influencing level and activity (Table 2). The main functional
386 locus modifying FXIII functions is the V34L [15, 111]. This SNP is the most widely investigated;
387 accordingly, many studies show L34 to enhance thrombin-dependent activation-rate of FXIII, because of
388 L34 renders the R37-G38 activation-site more available for thrombin [16, 17]. The above discussed
389 earlier FXIII activation-rate (Fig. 2) reflects in an altered cross-linked three-dimensional structure of the
390 fibrin network, more resistant to both endogenous and pharmacologic fibrinolysis, though different
391 fibrinogen concentrations have to be taken into consideration [112-114]. Though, controversal results,
392 large meta-analyses established a protective effect against venous and arterial thrombosis [115, 116].
393 The P564L, recently associated with decreased circulating levels, could reflect earlier tetramer
394 dissociation with effects on A-subunit degradation [15, 45, 47, 117-119]. The T204F, scarcely
395 investigated, was associated to decreased enzyme activity and levels [15, 47, 113, 118, 119]. Similarly,
396 the V650I and E651Q are very rare and less investigated compared to the V34L [15, 113, 117]. Table 2
397 summarizes the most important FXIIIA SNPs including those scarcely investigated in the clinical setting.
398 Finally, selective changes in the FXIII activation peptide sequence, may give back FXIII species with
399 increased activation rates potentially useful in designing and projecting of recombinant FXIIIA
400 molecules [120].

401 Besides the classical effects that FXIIIA SNPs may have on coagulation and related disorders
402 (i.e. hemorrhage and thrombosis), more interesting and less investigated aspects may come from new
403 and promising applications in the clinical practice.

404 Surgery, by definition, cuts the skin to reach the organ/tissue of interest. In the field of vascular
405 surgery, an interesting association has been demonstrated between healing time and FXIIIV34L after
406 superficial venous surgery [45]. Interestingly, the known genetic risk factor for iron overload in venous
407 leg ulcer (HFE C282Y) did not affect post-operative healing time once venous stasis was surgically
408 resolved regardless the presence of the protective genetic factor (FXIII V34L). In addition, the
409 polymorphic L34- and the L564-allele positively correlated with smaller lesion area both in single and
410 double heterozygosis in venous leg ulcer [47, 58]. These results ascribed significant smaller area to

lesions regardless the FXIIIA circulating level in patients, ascribing to the functional property of the SNP the primary effect on the extension of the skin lesion [43]. An attempt to create a DNA-array with prognostic purpose in the wound healing of chronic lesions confirmed these results also in combination with additional genes and SNPs [44]. Finally, additional precious clinical information might be drawn by this molecular tool such as the risk of establishment and the predictive early onset [58].

Another concrete example of FXIII pharmacogenetics comes from a large clinical trial in patients affected by eye diseases, genotyped for FXIIIA V34L. They were treated with photodynamic therapy with verteporfin (PDT-V) for severe macular degenerations complicated by choroidal neovascularization (CNV). The study aimed to define a pharmacogenetics correlation between this SNP and CNV responsiveness to either single PDT-V procedure [121-123] or to long-term PDT-V standardized as-needed protocol [124]. The therapeutic photo-thrombotic action, on which is based PDT-V [125-128], clearly indicates the presence of a high level of plausibility concerning the role of coagulation-balance SNPs and individual variable efficacy of PDT-V [129-132]. The beneficial effect is obtained with a laser-light-induced thrombosis of CNV, selectively photosensitized by verteporfin preferentially bounded to the endothelium of the aberrant neovessels in comparison with that of the normal microvascular networks. Post-PDT-V changes of CNV endothelium are induced by a photo-oxidative therapeutic occlusion of the neovascular network mediated by platelet activation and consequent release of vasoactive mediators. These molecules elicit a series of events (i.e. thrombosis, vasoconstriction, and increased vascular permeability), which finally lead to local hypoxia and CNV shutdown. Even though PDT-V has been initially considered an ideal treatment for neovascular macular degenerations [125], both persistence and extensiveness of CNV hemodynamic occlusion represent pivotal aspects for the assessment of therapeutic efficacy and, the early recanalization of the neovascular network, can be a key factor in determining poor responsiveness to photodynamic protocol [127, 128]. The unsatisfactory post-PDT-V result observed in the carriers of the "*hyperfibrinolytic*" L34-allele should be rationally related to a weak photo-thrombotic action within the CNV [121-124], similarly to what has been described also in patients taking aspirin during PDT-V protocol [133]. The locally PDT-V activated thrombosis [125] is responsible for the formation of a very different fibrin clot structures according to local fibrinogen levels

and FXIII genotype [112], with more loosely packed fibers and larger pores in patients with the polymorphic L34-allele. Accordingly, the L34-allele has been described as protective factor in patients with retinal artery occlusion [134] and retinal vein occlusion [135], as well as predisposing factor in individuals suffering from spontaneous sub-conjunctival hemorrhages [136, 137]. Similarly, the L34-allele was previously described having an opposite role in the cerebral stroke, being predisposing to primary intra-cerebral hemorrhage and protective against cerebral thrombotic ischemia [138].

Summarizing and Conclusions

The key role of FXIII in tissue regeneration has been known for decades, and its functions have been evaluated and investigated in several clinical settings. Tissue remodelling and wound healing are processes involving a complex series of steps finely tuned by a network of mutual reactions and feedbacks. Anomalous healing implies a dysregulation of the balance between the acute and early phases and the conclusive steps often leading to the establishment of chronic conditions responsible for poor tissue elasticity, loss of epithelial integrity, and excessive fibrosis. The final outcome of the perfectly concerted phases is important for both the healing of acute wounds and the healing under chronic inflammation status. FXIII effectively links the several components of the coagulation cascade to the process of the definite wound healing by means of non-enzymatic activities and chemical cross-linking of ECM proteins, cells and other constituents. Accordingly, FXIII takes part to cell proliferation, survival, and differentiation, crucial steps for optimal tissue regeneration. Accordingly, stem cells recruitment is strongly mediated and improved by tridimensional fibrin networks processed by FXIII. Because of the multitask properties of the FXIII molecule, and due to the fact that they can often act concomitantly and/or sequentially, a transient and acquired deficit of FXIII could establish, mainly due to its excessive consumption and this could affect the final healing process. Among these, chronic inflammation, bleeding episodes and activation of the coagulation cascade (i.e. thrombosis or infarction) can cause reduced levels of circulating FXIII. On these bases, the wound-healing effects of FXIII could be hampered in numerous clinical settings, including non-healing lesions, postsurgical conditions, inflammatory bowel disease, open heart surgery and infarction. FXIII levels often correlate inversely

with chronic and degenerative diseases activity (e.g. chronic venous ulcers or inflammatory bowel disease) and might negatively affect the clinical outcome in acute conditions (i.e. acute myocardial infarction). Accordingly, FXIII monitoring could give back information as healing biomarker, and exogenous FXIII supplementation could be suggested to improve tissue restitution and organ recovery of functions in a range of pathologic conditions in which a reduced FXIII availability has been established.

This review has dealt with and has summarized the roles and the multi-task properties and distinct functions that FXIII possesses articulating a new background on the future potential utility of FXIII as an adjuvant therapeutic agent or a useful prognostic bio/molecular marker in a variety of complex diseases in which effective coagulation activity and wound healing are involved.

Acknowledgements

The present paper was supported by financial funds from Italian Association against Thrombosis and cardiovascular disease (ALT).

References

1. Bagoly Z, Koncz Z, Harsfalvi J, et al. Factor XIII, clot structure, thrombosis. Thromb Res 2012; 129: 382–387.
2. Ichinose A. Factor XIII is a key molecule at the intersection of coagulation and fibrinolysis as well as inflammation and infection control. Int J Hematol 2012; 95: 362–370
3. Adány R. Intracellular factor XIII: cellular distribution of factor XIII subunit a in humans. Semin Thromb Hemost 1996;22(5):399-408.
4. Richardson VR, Cordell P, Standeven KF, Carter AM. Substrates of Factor XIII-A: roles in thrombosis and wound healing. Clin Sci (Lond) 2013;124(3):123-37.
5. Adány R, Kiss A, Muszbek L. Factor XIII: a marker of mono- and megakaryocytopoiesis. Br J Haematol 1987; 67: 167–72.
6. Katona É, Ajzner E, Tóth K, Kárpáti L, Muszbek L. Enzyme-linked immunosorbent assay for the determination of blood coagulation factor XIII A-subunit in plasma and in cell lysates. J Immunol Methods 2001; 258: 127–35.

492 7. Nurminskaya M, Kaartinen MT. Transglutaminases in mineralized tissues. Front Biosci 2006; 11:
493 1591–606.
494 8. Inbal A, Muszbek L, Lubetsky A, et al. Platelets but not monocytes contribute to the plasma levels of
495 factor XIII subunit A in patients undergoing autologous peripheral blood stem cell transplantation. Blood
496 Coagul Fibrinolysis 2004; 15: 249–53.
497 9. Cordell PA, Kile BT, Standeven KF, Josefsson EC, Pease RJ, Grant PJ. Association of coagulation
498 factor XIII-A with Golgi proteins within monocyte–macrophages: implications for subcellular trafficking
499 and secretion. Blood 2010; 115: 2674–81.
500 10. Ballerini G, Gemmati D, Moratelli S, Morelli P, Serino ML. A photometric method for the dosage of
501 factor XIII applied to the study of chronic hepatopathies. Thromb Res. 1995;78(5):451-6.
502 11. Adány R, Antal M. Three different cell types can synthesize factor XIII subunit A in the human liver.
503 Thromb Haemost 1996; 76: 74–9.
504 12. Souri M, Kaetsu H, Ichinose A. Sushi domains in the B subunit of factor XIII responsible for
505 oligomer assembly. Biochemistry 2008; 47: 8656–64.
506 13. Polgár J, Hidasi V, Muszbek L. Non-proteolytic activation of cellular protransglutaminase (placenta
507 macrophage factor XIII). Biochem J 1990; 267: 557–60.
508 14. Mosesson MW, Siebenlist KR, Hernandez I, Lee KN, Christiansen VJ, McKee PA. Evidence that
509 alpha2-antiplasmin becomes covalently ligated to plasma fibrinogen in the circulation: a new role for
510 plasma factor XIII in fibrinolysis regulation. J Thromb Haemost 2008;6(9):1565-70.
511 15. de Lange M, Andrew T, Snieder H, et al. Joint linkage and association of six single-nucleotide
512 polymorphisms in the factor XIII-A subunit gene, point to V34L as the main functional locus.
513 Arterioscler Thromb Vasc Biol 2006; 26: 1914-1919.
514 16. Kohler HP, Ariëns RA, Whitaker P, Grant PJ. A common coding polymorphism in the FXIII A-
515 subunit gene (FXIIIVal34Leu) affects cross-linking activity. Thromb Haemost 1998; 80: 704.
516 17. Ariëns RA, Philippou H, Nagaswami C, Weisel JW, Lane DA, Grant PJ. The factor XIII V34L
517 polymorphism accelerates thrombin activation of factor XIII and affects cross-linked fibrin structure.
518 Blood 2000; 96: 988-995.
519 18. Muszbek L, Bereczky Z, Bagoly Z, Komáromi I, Katona É. Factor XIII: a coagulation factor with
520 multiple plasmatic and cellular functions. Physiol Rev 2011;91(3):931-72.
521 19. Dickneite G, Herwald H, Korte W, et al. Coagulation factor XIII: a multifunctional transglutaminase
522 with clinical potential in a range of conditions. Thromb Haemost 2015; 113(4):686-97.
523 20. Karimi M, Bereczky Z, Cohan N, Muszbek L. Factor XIII deficiency. Semin Thromb Hemost 2009;
524 35: 426–38.
525 21. Ivaskevicius V, Seitz R, Kohler HP, et al. International registry on factor XIII deficiency: a basis
526 formed mostly on European data. Thromb Haemost 2007; 97: 914–21.

22. Peyvandi F, Palla R, Menegatti M, et al. European Network of Rare Bleeding Disorders Group. Coagulation factor activity and clinical bleeding severity in rare bleeding disorders: results from the European Network of Rare Bleeding Disorders. J Thromb Haemost 2012;10(4):615-21.
23. Board PG, Losowsky MS, Miloszewski KJ. Factor XIII: inherited and acquired deficiency. Blood Rev 1993; 7: 229–42.
24. Osaki T, Sugiyama D, Magari Y, Souri M, Ichinose A. Rapid immunochromatographic test for detection of anti-factor XIII A subunit antibodies can diagnose 90 % of cases with autoimmune haemorrhaphilia XIII/13. Thromb Haemost 2015;113:1347-56.
25. Muszbek L, Bagoly Z, Cairo A, Peyvandi F. Novel aspects of factor XIII deficiency. Curr Opin Hematol 2011; 18: 366–72.
26. Sharief LA, Kadir RA. Congenital factor XIII deficiency in women: a systematic review of literature. Haemophilia 2013;19(6):e349-57.
27. Inbal A, Muszbek L. Coagulation factor deficiencies and pregnancy loss. Semin Thromb Hemost 2003; 29: 171–4.
28. Ichinose A, Asahina T, Kobayashi T. Congenital blood coagulation factor XIII deficiency and perinatal management. Curr Drug Targets 2005; 6: 541–9.
29. Fisgin T, Yarali N, Kara A, et al. Hemostatic side effects of high-dose methotrexate in childhood acute lymphoblastic leukemia. Pediatr Hematol Oncol 2004;21(1):77-83
30. Ballerini G, Guerra S, Rodeghiero F, Castaman G. A contribution to the pathology of acquired plasma factor XIII deficiency. Semin Thromb Hemost 1985;11(4):357-61.
31. Song JW, Choi JR, Song KS, Rhee J-H. Plasma factor XIII activity in patients with disseminated intravascular coagulation. Yonsei Med J 2006; 47: 196–200.
32. Ogawa T, Morioka Y, Inoue T, Takano M, Tsuda S. Involvement of blood coagulation factor XIII in burn healing in the carbon tetrachloride-induced hepatic injury model in rats. Inflamm Res 1995; 44: 264–8.
33. Zeerleder S, Schroeder V, Lämmle B, Wuillemin WA, Hack CE, Kohler HP. Factor XIII in severe sepsis and septic shock. Thromb Res 2007; 119: 311–18.
34. Bockeria LA, Samsonova NN, Yurlov IA, et al. Dynamics of factor XIII levels after open heart surgery for congenital heart defects: do cyanotic and acyanotic patients differ? Pediatr Cardiol 2014;35(7):1108-15.
35. D'Argenio G, Cosenza V, Riegler G, Della Valle N, Deritis F, Mazzacca G. Serum transglutaminase correlates with endoscopic and histopathologic grading in patients with ulcerative colitis. Dig Dis Sci 2001; 46: 649–57.

36. Higaki S, Nakano K, Onaka S, et al. Clinical significance of measuring blood coagulation factor XIIIA regularly and continuously in patients with Crohn's disease. J Gastroenterol Hepatol 2006; 21: 1407–11.

37. Gemmati D, Zeri G, Orioli E, et al. Factor XIII-A dynamics in acute myocardial infarction: a novel prognostic biomarker? Thromb Haemost 2015;114(1):123-32.

38. National Hemophilia Foundation: MASAC Recommendations Concerning Products Licensed for the Treatment of Hemophilia and Other Bleeding Disorders. Available at: http://www.hemophilia.org/NHFWeb/MainPgs/MainNHF.aspx?menuid=57&contentid=693. Accessed 24 September, 2013.

39. Árokszállási A, Kerényi A, Katona É, et al. The use of recombinant factor XIII in a major bleeding episode of a patient with congenital factor XIII deficiency--the first experience. Haemophilia 2015;21(1):e118-21.

40. Inbal A, Oldenburg J, Carcao M, Rosholm A, Tehranchi R, Nugent D. Recombinant factor XIII: a safe and novel treatment for congenital factor XIII deficiency. Blood 2012; 31;119(22):5111-7

41. 7 Lovejoy AE, Reynolds TC, Visich JE, et al. Safety and pharmacokinetics of recombinant factor XIII-A2 administration in patients with congenital factor XIII deficiency. Blood 2006;108(1):57-62

42. Dorey E. First recombinant Factor XIII approved. Nat Biotechnol 2014; 32:210

43. Gemmati D, Tognazzo S, Serino ML, et al. Factor XIII V34L polymorphism modulates the risk of chronic venous leg ulcer progression and extension. Wound Repair and Regeneration 2004; 12:512-517.

44. Gemmati D, Federici F, Catozzi L, et al. DNA-array of gene variants in venous leg ulcers: Detection of prognostic indicators. Journal of Vascular Surgery 2009; 50:1444-1451.

45. Gemmati D, Tognazzo S, Catozzi L, et al. Influence of gene polymorphisms in ulcer healing process after superficial venous surgery. Journal of Vascular Surgery 2006; 44:554-562.

46. Peschen M, Thimm C, Weyl A, et al. Possible role of coagulation factor XIII in the pathogenesis of venous leg ulcers. Vasa 1998;27(2):89-93.

47. Tognazzo S, Gemmati D, Palazzo A, et al. Prognostic role of factor XIII gene variants in nonhealing venous leg ulcers. Journal of Vascular Surgery 2006; 44:815-819;

48. Raffetto JD. Inflammation in chronic venous ulcers. Phlebology. 2013;28 Suppl 1:61-7.

49. Zamboni P, Scapoli, Lanzara V, et al. Serum iron and matrix metalloproteinase-9 variations in limbs affected by chronic venous disease and venous leg ulcers. Dermatologic Surgery 2005; 31:644-649.

50. Singh AV, Subhashree L, Milani P, Gemmati D, Zamboni P. Interplay of iron metallobiology, metalloproteinases, and FXIII, and role of their gene variants in venous leg ulcer. Int J Low Extrem Wounds 2010;9(4):166-79.

51. Zamboni P, De Mattei M, Ongaro A, et al. Factor XIII contrasts the effects of metalloproteinases in human dermal fibroblast cultured cells. Vasc Endovascular Surg 2004; 38(5):431-8.

595 52. Zamboni P, Izzo M, Tognazzo S, et al. The overlapping of local iron overload and HFE mutation in
596 venous leg ulcer pathogenesis. Free Radic Biol Med 2006;40(10):1869-73.
597 53. Wozniak G, Noll T. Mechanisms influencing cellular physiology as a concept of treatment for wound
598 healing disturbances. Zentralbl Chir 2005; 130:526-33.
599 54. Hildenbrand T, Idzko M, Panther E, Norgauer J, Herouy Y. Treatment of nonhealing leg ulcers with
600 fibrin-stabilizing factor XIII: a case report. Dermatol Surg 2002;28:1098-9.
601 55. Wozniak G, Noll T. Factor XIII and wound healing. Hamostaseologie 2002;22:59-62.
602 56. Herouy Y, Hellstern MO, Vanscheidt W, Schöpf E, Norgauer J. Factor XIII-mediated inhibition of
603 fibrinolysis and venous leg ulcers. Lancet 2000;355:1970-71.
604 57. Wozniak G, Noll T, Brunner U, Hehrlein FW. Topical treatment of venous ulcer with fibrin
605 stabilizing factor: experimental investigation of effects on vascular permeability. Vasa 1999;28:160-3.
606 58. Zamboni P, Gemmati D. Clinical implications of gene polymorphisms in venous leg ulcer: a model
607 in tissue injury and reparative process. Thromb Haemost 2007;98:131-7.
608 59. Nahrendorf M, Swirski FK, Aikawa E, et al. The healing myocardium sequentially mobilizes two
609 monocyte subsets with divergent and complementary functions. J Exp Med 2007; 204: 3037-3047.
610 60. Panizzi P, Swirski FK, Figueiredo JL, et al. Impaired infarct healing in atherosclerotic mice with Ly-
611 6C(hi) monocytosis. J Am Coll Cardiol 2010; 55:1629–1638.
612 61. Swirski FK, Nahrendorf M. Leukocyte behavior in atherosclerosis, myocardial infarction, and heart
613 failure. Science 2013; 339: 161–166.
614 62. Nahrendorf M, Hu K, Frantz S, et al. Factor XIII deficiency causes cardiac rupture, impairs wound
615 healing, and aggravates cardiac remodeling in mice with myocardial infarction. Circulation 2006; 113:
616 1196–1202.
617 63. Nahrendorf M, Weissleder R, Ertl G. Does FXIII deficiency impair wound healing after myocardial
618 infarction? PLoS One 2006; 1: 48.
619 64. Nahrendorf M, Aikawa E, Figueiredo JL, et al. Transglutaminase activity in acute infarcts predicts
620 healing outcome and left ventricular remodelling: implications for FXIII therapy and antithrombin use in
621 myocardial infarction. Eur Heart J 2008; 29: 445–454.
622 65. Vanhoutte D, Heymans S. Factor XIII: the cement of the heart after myocardial infarction? Eur Heart
623 J 2008; 29: 427–428.
624 66. Mukherjee R, Zavadzkas JA, Saunders SM, et al. Targeted myocardial microinjections of a
625 biocomposite material reduces infarct expansion in pigs. Ann Thorac Surg 2008; 86:1268–1276.
626 67. Della Rocca DG, Willenberg BJ, Ferreira LF, et al. A degradable, bioactive, gelatinized alginate
627 hydrogel to improve stem cell/growth factor delivery and facilitate healing after myocardial infarction.
628 Med Hypotheses 2012; 79: 673–677.

629 68. Li XH, Zhou X, Zeng S, et al. Effects of intramyocardial injection of platelet-rich plasma on the
630 healing process after myocardial infarction. Coron Artery Dis 2008;19(5):363-70.
631 69. Wehberg KE, Answini G, Wood D, Todd J, Julian J, Ogburn N, Allen KB. Intramyocardial injection
632 of autologous platelet-rich plasma combined with transmyocardial revascularization. Cell Transplant
633 2009;18(3):353-9.
634 70. Mishra A, Velotta J, Brinton TJ, et al. RevaTen platelet-rich plasma improves cardiac function after
635 myocardial injury. Cardiovasc Revasc Med 2011;12(3):158-63.
636 71. Inbal A, Dardik R. Role of coagulation factor XIII (FXIII) in angiogenesis and tissue repair.
637 Pathophysiol Haemost Thromb 2006; 35: 162–165.
638 72. Chen YL, Sun CK, Tsai TH, et al. Adipose-derived mesenchymal stem cells embedded in platelet-
639 rich fibrin scaffolds promote angiogenesis, preserve heart function, and reduce left ventricular
640 remodeling in rat acute myocardial infarction. Am J Transl Res 2015;7(5):781-803.
641 73. Valgimigli M, Rigolin GM, Fucili A, et al. CD34+ and endothelial progenitor cells in patients with
642 various degrees of congestive heart failure. Circulation 2004;110(10):1209-12.
643 74. Sun CK, Zhen YY, Leu S, et al. Direct implantation versus platelet-rich fibrin-embedded adipose-
644 derived mesenchymal stem cells in treating rat acute myocardial infarction. Int J Cardiol
645 2014;173(3):410-23.
646 75. Kim JT, Chung HJ, Seo JY, et al. A fibrin-supported myocardial organ culture for isolation of
647 cardiac stem cells via the recapitulation of cardiac homeostasis. Biomaterials 2015;48:66-83.
648 76. Alkjaersig N, Fletcher AP, Lewis M, et al. Reduction of coagulation factor XIII concentration in
649 patients with myocardial infarction, cerebral infarction, and other thromboembolic disorders. Thromb
650 Haemost 1977; 38: 863–873.
651 77. Fletcher AP, Alkjaersig NK, Ghani FM, et al. Blood coagulation system pathophysiology in acute
652 myocardial infarction: the influence of anticoagulant treatment on laboratory findings. J Lab Clin Med
653 1979; 93: 1054–1065.
654 78. Kohler HP, Ariens RA, Catto AJ, et al. Factor XIII A-subunit concentration predicts outcome in
655 stroke subjects and vascular outcome in healthy, middle-aged men. Br J Haematol 2002; 118: 825–832.
656 79. Kucher N, Schroeder V, Kohler HP. Role of blood coagulation factor XIII in patients with acute
657 pulmonary embolism. Correlation of factor XIII antigen levels with pulmonary occlusion rate,
658 fibrinogen, D-dimer, and clot firmness. Thromb Haemost 2003; 90: 434–438.
659 80. Chatterjee T, Schroeder V, Windecker S, et al. Venous and intracoronary factor XIII A-subunit
660 antigen and activity levels are not associated with extent of coronary artery disease. J Thromb Haemost
661 2003; 1: 861–863.
662 81. Gemmati D, Federici F, Campo G, et al. Factor XIIIA-V34L and factor XIIIB-H95R gene variants:
663 effects on survival in myocardial infarction patients. Mol Med 2007; 13: 112–120.

82. Schroeder V, Ortner E, Mono ML, et al. Coagulation factor XIII activation peptide and subunit levels in patients with acute ischaemic stroke: a pilot study. Thromb Res 2010; 126: 122–127.
83. Ordás I, Eckmann L, Talamini M, Baumgart DC, Sandborn WJ. Ulcerative colitis. Lancet 2012; 380: 1606–19.
84. Baumgart DC, Sandborn WJ. Crohn's disease. Lancet 2012; 380: 1590–605.
85. Linskens RK, Van Bodegraven AA, Schoorl M, Tuynman HA, Bartels P. Predictive value of inflammatory and coagulation parameters in the course of severe ulcerative colitis. Dig Dis Sci 2001; 46: 644–8.
86. Chamouard P, Grunebaum L, Wiesel ML, et al. Significance of diminished factor XIII in Crohn's disease. Am J Gastroenterol 1998; 93: 610–14.
87. Vrij AA, Rijken J, Van Wersch JW, Stockbrügger RW. Differential behavior of coagulation factor XIII in patients with inflammatory bowel disease and in patients with giant cell arteritis. Haemostasis 2000; 29: 326–35.
88. Lorenz R, Olbert P, Born P. Factor XIII in chronic inflammatory bowel diseases. Semin Thromb Hemost 1996; 22: 451–5.
89. Lorenz R, Heinmüller M, Classen M, Tornieporth N, Gain T. Substitution of factor XIII: a therapeutic approach to ulcerative colitis. Haemostasis 1991; 21: 5–9.
90. Lorenz R, Born P, Olbert P, Classen M. Factor XIII substitution in ulcerative colitis. Lancet 1995; 345: 449–50.
91. Bregenzer N, Caesar I, Andus T, et al. Lack of clinical efficacy of additional factor XIII treatment in patients with steroid refractory colitis. The Factor XIII Study Group. Z Gastroenterol 1999; 37: 999–1004.
92. Kamata N, Oshitani N, Suekane T, et al. Combined use of factor XIII and endoscopic balloon dilatation in a patient with Crohn's disease, duodenal stenosis, and associated internal fistulas: the efficacy of coagulation factor XIII for the internal fistulas. Am J Gastroenterol 2008;103(6):1573-4.
93. Soendergaard C, Kvist PH, Seidelin JB, Nielsen OH. Tissue-regenerating functions of coagulation factor XIII. J Thromb Haemost 2013;11(5):806-16.
94. Loof TG, Mörgelin M, Johansson L, et al. Coagulation, an ancestral serine protease cascade, exerts a novel function in early immune defense. Blood 2011;118(9):2589-98.
95. Hoppe B. Fibrinogen and factor XIII at the intersection of coagulation, fibrinolysis and inflammation. Thromb Haemost 2014;112(4):649-58.
96. Moussata D, Goetz M, Gloeckner A, et al. Confocal laser endomicroscopy is a new imaging modality for recognition of intramucosal bacteria in inflammatory bowel disease in vivo. Gut 2011; 60: 26–33.

698 97. Rea CJ, Foley JH, Sørensen B. Factor XIII in the treatment of hemophilia A. N Engl J Med
699 2012;366(3):281-3.
700 98. Rea CJ, Foley JH, Okaisabor O, Sørensen B. FXIII: mechanisms of action in the treatment of
701 hemophilia A. J Thromb Haemost 2014;12(2):159-68.
702 99. Rea CJ, Foley JH, Ingerslev J, Sørensen B. Factor XIII combined with recombinant factor VIIa: a
703 new means of treating severe hemophilia A. J Thromb Haemost 2011;9(3):510-6.
704 100. Brummel-Ziedins KE, Branda RF, Butenas S, Mann KG. Discordant fibrin formation in
705 hemophilia. J Thromb Haemost 2009;7(5):825-32.
706 101. Johansson PI, Jacobsen N, Viuff D, et al. Differential clot stabilising effects of rFVIIa and rFXIII-
707 A2 in whole blood from thrombocytopenic patients and healthy volunteers. Br J Haematol
708 2008;143(4):559-69.
709 102. American Heart Association. Cardiovascular Disease Statistics. Available at:
710 http://www.americanheart.org/presenter.jhtml?identifier_4478. Accessed August 20, 2009.
711 103. National Heart, Lung, and Blood Institute. NHLBI Financial Year 2008 Fact Book. Available at:
712 http://www.nhlbi.nih.gov/about/factbook/toc.htm. Accessed August 20, 2009.
713 104. Nahrendorf M. Imaging of infarct healing predicts left ventricular remodeling and evolution of
714 heart failure: focus on protease activity. Circ Cardiovasc Imaging 2011;4(4):351-3.
715 105. van der Laan AM, Nahrendorf M, Piek JJ. Healing and adverse remodelling after acute myocardial
716 infarction: role of the cellular immune response. Heart 2012;98(18):1384-90.
717 106. Visich JE, Zuckerman LA, Butine MD, et al. Safety and pharmacokinetics of recombinant factor
718 XIII in healthy volunteers: a randomized, placebo-controlled, double-blind, multi-dose study. Thromb
719 Haemost 2005;94(4):802-7.
720 107. Reynolds TC, Butine MD, Visich JE, et al. Safety, pharmacokinetics, and immunogenicity of
721 single-dose rFXIII administration to healthy volunteers. J Thromb Haemost 2005;3(5):922-8.
722 108. Board PG, Webb GC, McKee J, Ichinose A. Localization of the coagulation factor XIII A subunit
723 gene (F13A) to chromosome bands 6p24-p25. Cytogenet Cell Genet 1988; 48: 25–27.
724 109. Ichinose A, Davie EW. Characterization of the gene for the A subunit of human factor XIII (plasma
725 transglutaminase), a blood coagulation factor. Proc Natl Acad Sci USA 1988; 85: 5829–5833.
726 110. Saha N, Aston CE, Low PS, Kamboh MI. Racial and genetic determinants of plasma factor XIII
727 activity. Genet Epidemiol 2000;19(4):440-55.
728 111. Mikkola H, Syrjälä M, Rasi V, et al. Deficiency in the A-subunit of coagulation factor XIII: two
729 novel point mutations demonstrate different effects on transcript levels. Blood 1994;84(2):517-25.
730 112. Ariëns RA, Lai TS, Weisel JW, Greenberg CS, Grant PJ. Role of factor XIII in fibrin clot formation
731 and effects of genetic polymorphisms. Blood 2002;100(3):743-54.

732 113. Anwar R, Gallivan L, Edmonds SD, Markham AF. Genotype/phenotype correlations for
733 coagulation factor XIII: specific normal polymorphisms are associated with high or low factor XIII
734 specific activity. Blood 1999;93(3):897-905.
735 114. Marín F, González-Conejero R, Lee KW, et al. A pharmacogenetic effect of factor XIII valine 34
736 leucine polymorphism on fibrinolytic therapy for acute myocardial infarction. J Am Coll Cardiol
737 2005;45(1):25-9.
738 115. Wells PS, Anderson JL, Scarvelis DK, Doucette SP, Gagnon. Factor XIII Val34Leu variant is
739 protective against venous thromboembolism: a huge review and meta-analysis. Am J Epidemiol 164:
740 101–109, 2006.
741 116. Voko Z, Bereczky Z, Katona E, Adany R, Muszbek L. Factor XIII Val34Leu variant protects
742 against coronary artery disease. A meta-analysis. Thromb Haemost 97: 458–463, 2007.
743 117. Heng CK, Lal S, Saha N, Low PS, Kamboh MI. The impact of factor XIIIa V34L polymorphism on
744 plasma factor XIII activity in the Chinese and Asian Indians from Singapore. Hum Genet
745 2004;114(2):186-91.
746 118. Ruigrok YM, Slooter AJ, Rinkel GJ, Wijmenga C, Rosendaal FR. Genes influencing coagulation
747 and the risk of aneurysmal subarachnoid hemorrhage, and subsequent complications of secondary
748 cerebral ischemia and rebleeding. Acta Neurochir (Wien). 2010;152(2):257-62.
749 119. Pruissen DM, Slooter AJ, Rosendaal FR, van der Graaf Y, Algra A. Coagulation factor XIII gene
750 variation, oral contraceptives, and risk of ischemic stroke. Blood 2008;111(3):1282-6.
751 120. Jadhav MA, Isetti G, Trumbo TA, Maurer MC. Effects of introducing fibrinogen Aalpha character
752 into the factor XIII activation peptide segment. Biochemistry 2010;49(13):2918-24.
753 121. Parmeggiani F, Costagliola C, Gemmati D, et al. Predictive role of coagulation-balance gene
754 polymorphisms in the efficacy of photodynamic therapy with verteporfin for classic choroidal
755 neovascularization secondary to age- related macular degeneration. Pharmacogenet Genomics 2007; 17:
756 1039-1046.
757 122. Parmeggiani F, Costagliola C, Gemmati D, et al. Coagulation gene predictors of photodynamic
758 therapy for occult choroidal neovascularization in age-related macular degeneration. Invest Ophthalmol
759 Vis Sci 2008; 49: 3100-3106.
760 123. Parmeggiani F, Gemmati D, Costagliola C, et al. Impact of coagulation-balance gene predictors on
761 efficacy of photodynamic therapy for choroidal neovascularization in pathologic myopia.
762 Ophthalmology 2010; 117: 517-523.
763 124. Parmeggiani F, Costagliola C, Semeraro F, et al. Effect of factor XIII-A G185T polymorphism on
764 visual prognosis after photodynamic therapy for neovascular macular degeneration. Int J Mol Sci 2015;
765 16: 19796-19811.

766 125. Schmidt-Erfurth U, Hasan T. Mechanisms of action of photodynamic therapy with verteporfin for
767 the treatment of age-related macular degeneration. Surv Ophthalmol 2000; 45: 195-214.
768 126. Schmidt-Erfurth U, Michels S, Barbazetto I, Laqua H. Photodynamic effects on choroidal
769 neovascularization and physiological choroid. Invest Ophthalmol Vis Sci 2002; 43: 830-841.
770 127. Michels S, Schmidt-Erfurth U. Sequence of early vascular events after photodynamic therapy.
771 Invest Ophthalmol Vis Sci 2003; 44: 2147-2154.
772 128. Schmidt-Erfurth U, Niemeyer M, Geitzenauer W, Michels S. Time course and morphology of
773 vascular effects associated with photodynamic therapy. Ophthalmology 2005; 112: 2061-2069.
774 129. Parmeggiani F, Gemmati D, Costagliola C, Sebastiani A, Incorvaia C. Predictive role of gene
775 polymorphisms affecting thrombin-generation pathway in variable efficacy of photodynamic therapy for
776 neovascular age-related macular degeneration. Recent Pat DNA Gene Seq 2009; 3: 114-122.
777 130. Parmeggiani F, Gemmati D, Costagliola C, Sebastiani A, Incorvaia C. Predictive role of C677T
778 MTHFR polymorphism in variable efficacy of photodynamic therapy for neovascular age-related
779 macular degeneration. Pharmacogenomics 2009; 10: 81-95.
780 131. Parmeggiani F, Costagliola C, Incorvaia C, Sebastiani A, Gemmati D. Pharmacogenetic aspects in
781 therapeutic management of subfoveal choroidal neovascularisation: role of factor XIII-A 185 T-allele.
782 Curr Drug Targets 2011; 12: 138-148.
783 132. Parmeggiani F, Gemmati D, Costagliola C, et al. Genetic predictors of response to photodynamic
784 therapy. Mol Diagn Ther 2011; 15: 195-210.
785 133. Ranchod TM, Guercio JR, Ying GS, Brucker AJ, Stoltz RA. Effect of aspirin therapy on
786 photodynamic therapy with verteporfin for choroidal neovascularization. Retina 2008; 28: 711-716.
787 134. Weger M, Renner W, Stanger O, et al. Role of factor XIII Val34Leu polymorphism in retinal artery
788 occlusion. Stroke 2001;32(12):2759-61
789 135. Yioti GG, Panagiotou OA, Vartholomatos GA, et al. Genetic polymorphisms associated with retinal
790 vein occlusion: a Greek case-control study and meta-analysis. Ophthalmic Genet 2013;34(3):130-9.
791 136. Incorvaia C, Costagliola C, Parmeggiani F, Gemmati D, Scapoli GL, Sebastiani A. Recurrent
792 episodes of spontaneous subconjunctival hemorrhage in patients with factor XIII Val34Leu mutation.
793 Am J Ophthalmol 2002;134(6):927-9.
794 137. Parmeggiani F, Costagliola C, Incorvaia C, et al. Prevalence of factor XIII Val34Leu polymorphism
795 in patients affected by spontaneous subconjunctival hemorrhage. Am J Ophthalmol
796 2004;138(3):481-4.
797 138. Gemmati D, Serino ML, Ongaro A, et al. A common mutation in the gene for coagulation factor
798 XIII-A (VAL34Leu): a risk factor for primary intracerebral hemorrhage is protective against
799 atherothrombotic diseases. Am J Hematol 2001;67(3):183-8.