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Role of glycoprotein VI (GPVI) in platelet interaction with fibrinogen and fibrin in arterial thrombosis

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Table of Content

Abbreviations and acronyms

ACS	acute coronary syndrome
ADAM	a disintegrin and metalloproteinase domain containing protein
ADP	adenosine diphosphate
ASA	acetylsalicylic acid, Aspirin ®
Btk	Bruton's tyrosine kinase
Ca^{2+}	calcium
CRP	collagen-related peptide
CYP 450	cytochrome P450
DC	dendritic cell
DAPT	dual anti-platelet therapy
DEP	diesel exhaust particles
EC	endothelial cell
ELISA	enzyme-linked immunosorbent assay
Et al.	et alii [lat.], "and others"
F	coagulating factor
FcRγ	fragment crystallizable receptor γ-chain
GP	glycoprotein
GPCR	G protein- coupled receptor
GPRP	Gly-Pro-Arg-Pro
GPVI-Fc	recombinant dimeric GPVI-Fc fusion protein
GPVI-His	histidine- tagged GPVI
GPO	glycine-proline-hydroxyproline
GPIIb-IIIa	Glycoprotein IIb-IIIa (Integrin αIIbβ3)
GPIa-IIa	Glycoprotein Ia-IIa (Integrin α2β1)
Hom	homozygous
ICAM-1	intercellular adhesion molecule-1
Ig	immunoglobulin
IL	interleukin
ITAM	immunoreceptor tyrosine-based activation motif
K_D	dissociation constant
kDa	kilodalton
LAT	linker for activation of T cells
LDL	low-density lipoprotein
LSARLAF	Leu-Ser-Ala-Arg-Leu-Ala-Phe

MCP-1	monocyte chemotactic protein- 1
MMP	matrix metalloproteinases
mRNA	messenger ribonucleic acid
oxLDL	oxidized low-density lipoprotein
PCI	percutaneous coronary intervention
PDI	protein disulfide-isomerase
PLCγ2	phospholipase Cγ2
PI3Kβ	phosphatidylinositol 3-kinase-β
PS	phosphatidylserine
/s	per second
Ser	serine
sGPVI	soluble GPVI
SH2 domain	Src 2 homology domain
SIM	structured illumination microscopy
SLP76	lymphocyte cytosolic protein 2
Src	sarcoma kinase
Syk	spleen tyrosine kinase
TF	tissue factor
TFPI	tissue factor pathway inhibitor
TXA2	thromboxane A_2
TNF-α	tumor necrosis factor
VSMC	vascular smooth muscle cells VSMC
VCAM-1	vascular adhesion molecule-1
VWF	von Willebrand factor

Publication list

- Ebrahim M, Jamasbi J, Adler K, Megens RTA, M'Bengue Y, Blanchet X, Uhland K, Ungerer M, Brandl R, Weber C, Elia N, Lorenz R, Münch G, Siess W. **Dimeric Glycoprotein VI Binds to Collagen but Not to Fibrin.** Thromb Haemost. 2018 Feb;118(2):351-361. doi: 10.1160/TH17-04-0302. Epub 2018 Jan 29. PMID: 29378359.

- Zhang D, Ebrahim M, Adler K, Blanchet X, Jamasbi J, Megens RTA, Uhland K, Ungerer M, Münch G, Deckmyn H, Weber C, Elia N, Lorenz R, Siess W. **Glycoprotein VI is not a Functional Platelet Receptor for Fibrin Formed in Plasma or Blood.** Thromb Haemost. 2020 Jun;120(6):977-993. doi: 10.1055/s-0040-1710012. Epub 2020 Jun 3. PMID: 32492725.

Due to copyright reasons the author was not granted permission to attach the above-mentioned publications to this dissertation.

Please note: Throughout the following introductory paragraphs passages from the above-mentioned publications can be found. Adoption of the passages (written by the author) was conducted with the consent of all co-authors of both publications and Georg Thieme Verlag KG.

Introduction

1.1 Primary and secondary hemostasis

Under physiological conditions, blood flows through the vascular system without penetration of the vascular wall. Traumatic injury or pathologic alterations (e.g. atherosclerosis) to the vessel wall compromise the integrity of the endothelium which in turn triggers a series of events: Firstly, bleeding into the tissue occurs and subendothelial extracellular matrix (containing collagen, von Willebrand factor (VWF), laminin, fibronectin and thrombospondin) is exposed to the bloodstream. To minimize bleeding, thrombocytes, together with soluble plasma components, form a clot to block further leakage.[1] This mechanism is called hemostasis and can be sectioned into primary and secondary hemostasis.

During primary hemostasis, thrombocytes form a clot over the vascular lesion by first adhering to the vessel wall[2] and then acquiring further thrombocytes to create a temporary clot, termed aggregation (primary hemostatic thrombus).[3] The primary hemostatic thrombus develops rapidly but lacks firm adhesion. A more robust thrombus develops during secondary hemostasis. By activation of the intrinsic and extrinsic pathways of the coagulation cascade, activated coagulating factors are generated in a defined chronology which leads to the formation of fibrin fibers. Fibrin fibers stabilize the clot and initiate the healing process, while fibrinolytic agents (antithrombin III, protein C and S, and tissue factor pathway inhibitor (TFPI)) simultaneously regulate hemostasis.

These algorithms are crucial to the prevention of trauma-associated blood loss. However, unrestrained platelet aggregation in diseased vessels can cause vascular thrombosis, which in turn results in myocardial infarction, stroke or other related conditions (see 3.2. Atherosclerosis and atherothrombosis).

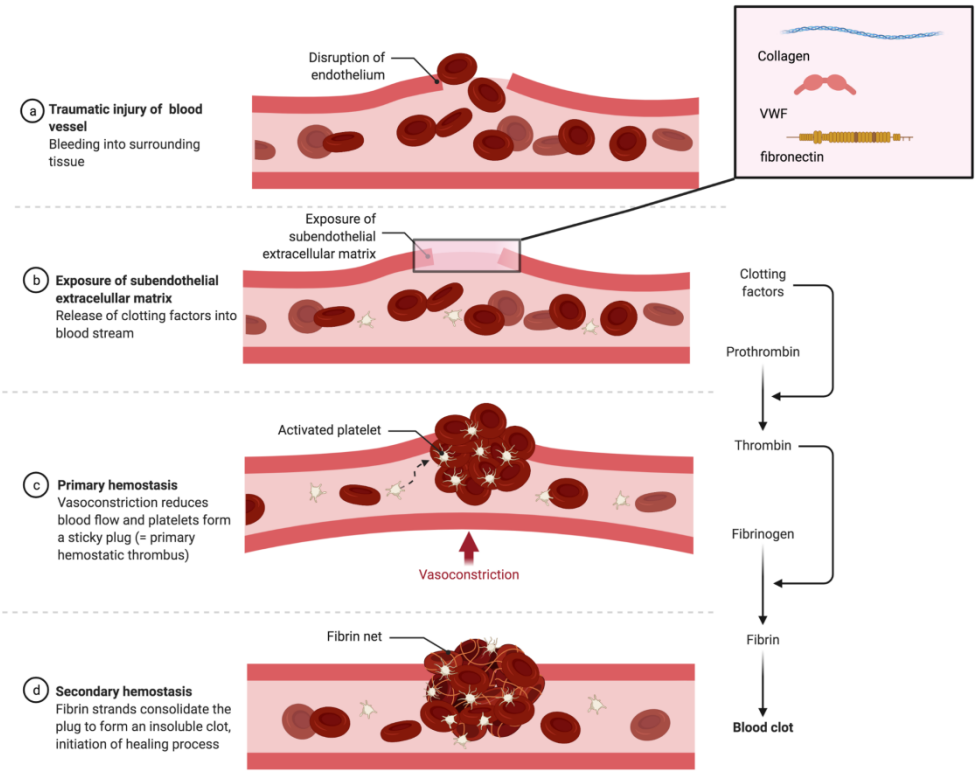

Figure 1: Primary and secondary hemostasis is initiated after traumatic injury of the vascular wall. *Created with BioRender.com.*[4]

1.1.1 Platelets and platelet receptors

Thrombocytes, also referred to as platelets, like other blood cells of the myeloid cell line (erythrocytes, leukocytes), originate from the bone marrow, where they are formed from megakaryocytes.[5] With a diameter of 2-4µm, platelets are the smallest corpuscular blood cells. Platelets lack a cell nucleus and therefore have restricted ability to synthesize protein[6]- a phenomenon, which plays an essential role in pharmacological inhibition of platelet activation. Under healthy conditions, the average platelet count of an adult lies between 150 000- 450 000/µl. The physiological lifetime of thrombocytes is 7-10 days with a daily regeneration rate of approximately 20%.

When activated, thrombocytes undergo a shape change.[7,8] Then the average surface is altered from approximately 8µm^2 to 13µm^2 by the formation of finger-shaped extensions of the plasma membrane, called pseudopodia.[9]

Thrombocytes play a crucial role in primary and secondary hemostasis.[3,10] In a first step, platelets decelerate and adhere to the extracellular matrix of the injured vascular wall ("tethering").[11] Platelet adhesion is primarily, among others, mediated by the interplay of von Willebrand factor (VWF) with the platelet glycoprotein GPIb-V-IX complex.[12-16] This

interaction is characterized by a rapid “on-off-rate”, which leads to a transient but less firm adhesion of thrombocytes.[17] VWF is a multimeric adhesive glycoprotein which has binding sites for collagen,[18] GP Ib[19,20] and integrin αIIbβ3.[21] VWF plays a major role under conditions with high shear rate as found in arterioles and arterial stenosis e.g. due to atherosclerosis.[14,22]

The transient adhesion allows for stable binding of platelets by the collagen receptors glycoprotein VI (GPVI) and integrin $\alpha_2\beta_1$, and therefore spreading of adhering platelets.
While integrin $\alpha_2\beta_1$ is primarily important for adhesion of platelets to collagen, GPVI was found to be relevant as a signaling receptor in the activation of platelets by collagen.[23,24] Binding of GPVI to its ligands leads to a shift of $\alpha_2\beta_1$ and $\alpha IIb\beta_3$ integrin on the platelet surface from a low- to a high- affinity state, which enables further ligand binding.[25,26]

GPVI signaling leads mainly to the release of thromboxane A_2 (TXA_2) and secretion of granule contents such as adenosine diphosphate (ADP)[3,27,28]. Both function as secondary positive-feedback mediators; ADP promotes further platelet activation via G protein–coupled receptors (GPCRs) P_2Y_1[29] and P_2Y_{12}[30]. Thromboxane A2[31] derives from arachidonic acid, which is converted by cyclooxygenase-1 and thromboxane- synthase and activates the GPCRs, TPα and TPβ, on the platelet surface.[32]
Activation of integrin αIIbβ3 leads to binding of fibrinogen and in turn to platelet aggregation.[33] Unlike integrin α2β1, αIIbβ3 is crucial for hemostasis. Genetic deficiency or dysfunction of this integrin results in impaired adhesion and absence of aggregation and underlies Glanzmann thrombasthenia, an inherited autosomal recessive bleeding disorder.[34]

Besides the collagen-induced activation of platelets[35] through exposure of extracellular matrix within the vascular wall, platelets can be activated by thrombin, triggered by the stimulation of coagulation factors such as tissue factor (TF).[36] Tissue factor, a 47 kDa membrane-associated protein, is expressed on various cells of the arterial wall, including fibroblasts, VSMCs and monocytes, where its secretion can be stimulated chemically.[37,38] It is unclear whether functionally significant amounts of the protein can be found on platelets.[39]
When TF comes into contact with plasma FVIIa, the coagulation cascade is initiated, resulting in the formation of thrombin which stimulates both platelets and fibrin formation to develop into a platelet-rich thrombus. Thrombin stimulated platelet activation can occur independently of endothelium disruption, VWF[40] or GPVI[41].

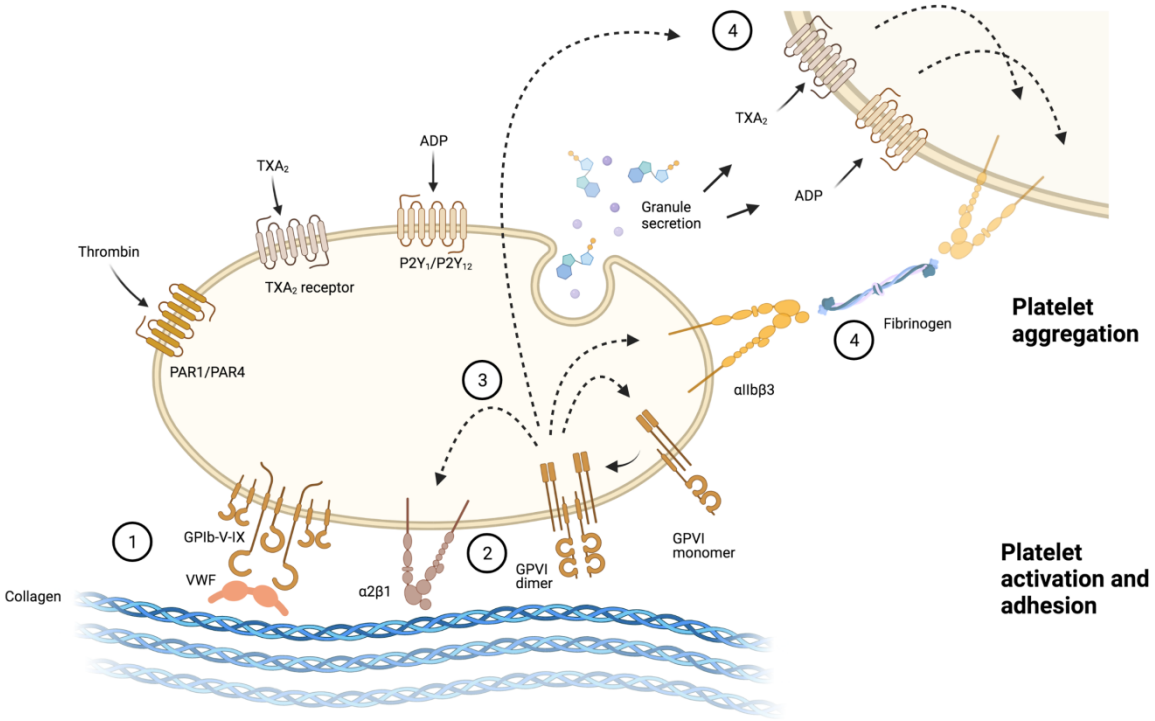

Figure 2: Mechanisms of platelet activation, adhesion and aggregation on collagen after injury to the vascular endothelium.
1. Platelets decelerate and adhere to the extracellular matrix of the injured vascular wall mediated by transient interaction of the GPIb-V-IX complex and immobilized VWF on collagen. **2.** Stable adhesion of platelets via binding of integrin $\alpha 2\beta 1$ and GPVI to collagen. During platelet activation, GPVI dimers are formed from monomers. **3.** GPVI-dependent signal transduction initiates platelet activation. "Inside-out-signaling" of integrin $\alpha 2\beta 1$ and αIIbβ3 leads to a shift from a low- to a high- affinity state, and release of secondary agonists ADP and TXA_2. **4.** Binding of fibrinogen via integrin αIIbβ3 results in platelet aggregation. ADP and TXA_2 promote further activation of platelets via stimulation of $P2Y_{12}$- and TXA_2-receptors. GPVI= glycoprotein VI; ADP= adenosine diphosphate; TXA2= thromboxane A_2, VWF = von Willebrand factor. *Created with BioRender.com*[42] *and modified with permission from Jamasbi et al*[43].

1.1.2 Coagulation cascade: Generation of fibrin

In the human organism, coagulation comprises both cellular (platelets) and soluble (proteins) elements. During secondary hemostasis, cross-linked fibrin is produced by means of a coagulation cascade involving two separate, initial pathways. Fibrin, as mentioned earlier, is necessary to form a stable blood clot after vessel wall injury. The two pathways are the contact activation pathway (intrinsic pathway)[44] and the tissue factor pathway (extrinsic pathway, see above)[45], the latter being the more dominant pathway.[46] Most coagulating factors (F) are serine proteases that cleave downstream proteins. On the contrary, tissue factor (FIII), FV, FVIII are categorized as glycoproteins and FXIII is a transglutaminase. All coagulation factors circulate as inactive enzyme precursors (zymogens); their activation is indicated by an additional “a” added to the Roman numbering.[47,48]

A thorough explanation of the cascade is far beyond the scope of this introductory paragraph. Instead, a simplified diagram will be provided (see Figure 3). Furthermore, it is crucial to understand that the distinction between the two pathways is entirely arbitrary and relies on laboratory testing.[49] The role of the contact activation pathway is still under debate. While some data suggests that it plays a more prominent role in initiating clot formation[50], elsewhere it is claimed to be more relevant in congenital immunity and inflammatory processes.[51]

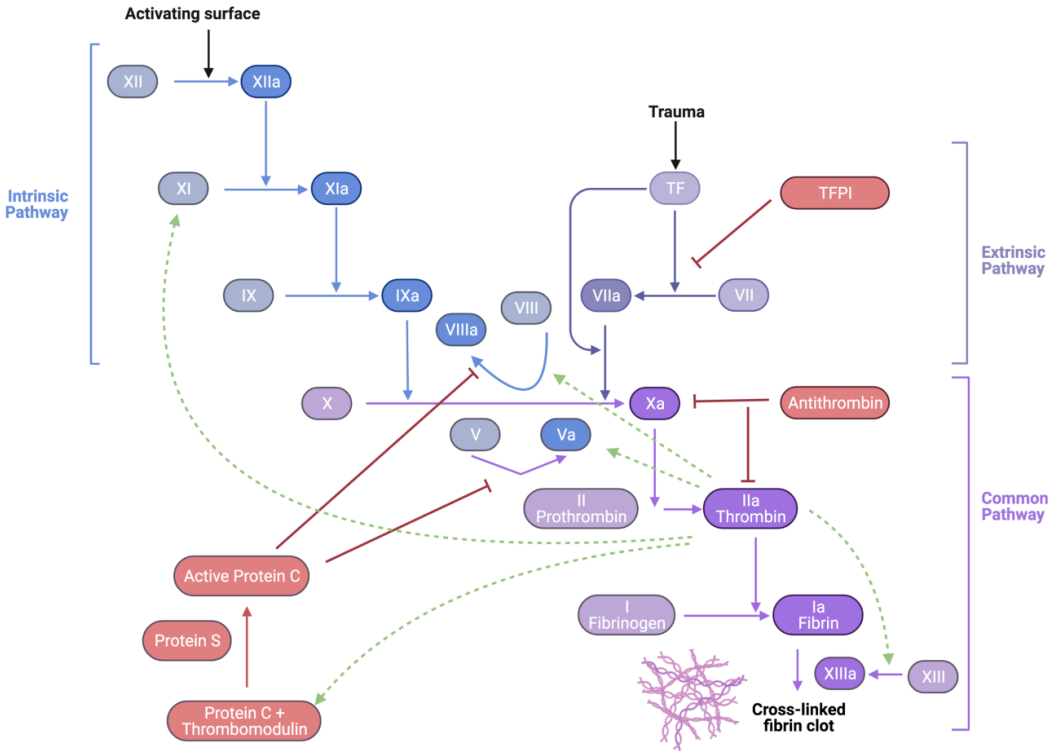

Figure 3: Coagulation cascade.
Tissue factor pathway (extrinsic) 1. TF forms a complex with circulating factor FVIIa, which activates FIX and FX. **2.** FVII is activated by thrombin, FXIa, FXII, and FXa. **3.** Tissue factor pathway inhibitor (TFPI) inhibits activation of FX by means of TF-FVIIa. **4.** FXa and FVa form the "prothrombinase complex" to transform prothrombin into thrombin. **5.** Thrombin is generated and has the highest positive feedback potential. It activates FV and FVIII (which forms a complex with FIX). **6.** FVIIIa and FIXa build the tenase complex and again activate FX.[48,52]
Contact activation pathway (intrinsic) 1. The primary complex is formed on collagen by high-molecular- weight kininogen (HMWK)[53], prekallikrein and FXII (Hageman factor). [54-56] **2.** Prekallikrein is converted to kallikrein and FXII is activated. **3.** FXIIa converts FXI to FXIa, which activates FIX[57]. **4.** FIXa, with its co-factor FVIIIa, forms the tenase complex, which converts FX to FXa. Both pathways merge to become the final common pathway[58,59], in which thrombin converts fibrinogen into fibrin and activates FV, FVIII and FXIII.[47] Continued activation of FVIII and FIX promotes the prothrombotic state of the coagulation cascade. Regulation to prevent overstimulation and thus pathologic blood clotting is secured by the anticoagulant pathways including, amongst others, protein C and S, thrombomodulin, tissue factor pathway inhibitor, and antithrombin.[48] *Created with BioRender.com.*[60]

In the final steps of the coagulation cascade, the insoluble polymer fibrin is formed from fibrinogen by thrombin-induced proteolytic cleavage. Fibrinogen has a molecular weight of 340 kDa and comprises two repeats of three polypeptides: the Aα, Bβ and γ chains.[61] These six chains form a dimeric structure with a central E-region flanked by two D-regions[62] on each side. Cleavage of fibrinogen at distinct sites of the central E domain at the N-termini of the α and the β chains result in the release of fibrinopeptides A and B.[63] Hence, polymerization sites are exposed and interact with the D-region of adjacent fibrinogen molecules.[64] FXIIIa initiates cross-linking of fibrin fibrils, thereby increasing clot stability and resistance to fibrinolysis.[65,66]

1.2 Atherosclerosis and atherothrombosis

Atherosclerosis is a systemic arterial disease characterized by local lipid accumulation in the intima, which leads to inflammation, smooth muscle cell proliferation, fibrous matrix accumulation, and plaque formation.[67] After erosion or rupture of an atherosclerotic plaque, uncontrolled platelet deposition can occur and result in partially or totally occlusive arterial thrombosis - referred to as **atherothrombosis**. Its primary clinical manifestations are ischemic heart disease[68], ischemic stroke, and peripheral arterial disease[69].

Up to this day, cardiovascular disease is the leading cause of death and long-term morbidity.[70] Although cardiovascular death rates have significantly decreased in developed countries, rates have been rising in developing countries, with now about 80% of the burden localized in low- to middle-income countries. In developed countries - despite optimal treatment involving highest technology and secondary prevention therapies - recurrent events are reported in 10% of all patients in the first 12 months following an acute coronary syndrom.[71] According to the Global Burden of Diseases, Injuries, and Risk Factor Study 2015, cardiovascular disease affected about 422 million people and caused an estimated 17.9 million deaths worldwide in 2015, hence 31% of all global deaths. Epidemiologists estimate that by 2030, approximately 23.6 million people will die each year as a consequence of cardiovascular diseases.[72]

The complex **mechanisms of atherosclerosis** and atheroprogression are still not completely understood. Despite controversial findings, the hypothesis of "response-to-injury"[73,74] appears to be widely accepted. Furthermore, atherosclerosis is considered a chronic inflammatory disease as signs of inflammation can be found throughout all stages of atheroprogression.[75]

In early-stage atherosclerosis, endothelial injury, hemodynamic turbulances[76], and abnormal lipid metabolism[67] mediate the atherogenic process and lead to inflammatory changes in endothelial cells (ECs). Activated ECs attract cells of the immune system (lymphocytes and monocytes) by displaying various signaling proteins on their surface, among others, monocyte chemoattractant protein-1 (MCP-1)[77,78], interleukin-8 (IL-8)[79], vascular adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1)[80], E-selectin and P-selectin.[81,82] Leukocytes and monocytes[83] adhere to the endothelial cells, infiltrate the arterial wall and cause inflammation which results in further endothelial dysfunction. Various cells and cytokines facilitate the inflammatory process, including lymphocytes (T and B cells), macrophages, dendritic cells (DCs), vascular smooth muscle cells (VSMCs), ILs and tumor necrosis factor (TNF-α).

Lipoproteins, foremost LDL (low-density lipoprotein), accumulate in the intimal layer of the vessel wall; oxidative stress leads to the production of oxidized LDL (oxLDL)[84] which

stimulates secretion of cytokines, chemokines and growth factors. Monocytes differentiate into macrophages and by ingestion oxLDL[85] transform into foam cells. VSMCs can degenerate into macrophage-like cells, which can- by overconsumption of lipoproteins- turn into foam cells, like other macrophages.[86] Foam cells typically occur in the "fatty streak", an early, primarily asymptomatic sign of atherosclerosis.

In late-stage atherosclerosis, macrophages secrete matrix metalloproteinases (MMPs),[87] which degrade collagen fibers,[88,89] allowing increased platelet adhesion onto these modified fibers.[90,91] Further secretion of chemokines and growth factors stimulates proliferation of VSMCs, building the fibroatheroma.
In contrast to the physiological activation of platelets during primary hemostasis due to vessel wall injury, plaque rupture or –erosion[92] can lead to intraluminal thrombosis, a pathological process termed **atherothrombosis.** After rupture of an atherosclerotic plaque, plaque components, including highly thrombogenic fibrillar collagen I and III and plaque tissue factor, are exposed to the bloodstream. Other compounds like VWF, laminin, fibronectin, vitronectin, thrombospondin, fibrinogen, fibrin, lysophosphatidic acid, and oxLDL stimulate further platelet adhesion and activation.[93,94] Studies have shown that atherosclerotic arteries have a much higher thrombogenic potential compared to healthy vessels. One explanation may be the modification of collagen I and III. In plaque lesions, the latter are degraded by matrix-metalloprotease -2,[95] which entails a drastically increased platelet response.[90]

Stable atherosclerotic plaques typically exhibit a solid fibrous cap with large quantities of VSMCs and extracellular matrix and a relatively small prothrombogenic, lipid rich core[96]. The fibrous cap prevents exposure of the core to the bloodstream: "*The thinner the fibrous cap, the higher the risk of plaque rupture*".[97] Robustness of an atherosclerotic plaque is determined by tissue composition[36,98] rather than the extent of luminal stenosis.[97,99-101] Destabilization of the atherosclerotic plaque underlies various mechanisms. T-cell mediated Interferon-γ secretion diminishes the synthesis of collagen I and III by VSMCs, and extracellular matrix is degraded involving formation of MMPs by stimulated macrophages.[88,89,102] Plaque rupture is estimated to be accountable for 60-75% of all acute coronary syndromes.

1.3 Antiplatelet agents

Antiplatelet therapy is one of the main therapeutic caterpillars for patients with acute coronary syndrome planned for percutaneous coronary intervention and represents a key measure in the primary and secondary prevention of cardiovascular events. Antiplatelet agents target either enzymes or platelet receptors essential to the development of arterial thrombosis.

Low-dose **acetylsalicylic acid (Aspirin®, ASA),** an irreversible cyclooxygenase inhibitor, is a well-established antiplatelet agent. Via acetylation of a serine residue (Ser529) of cyclooxygenase 1 and 2, ASA impairs the synthesis of prostaglandin G2 and H2, leading to an impaired generation of thromboxane A2 for the platelet lifetime. Subsequently, platelet activation through the thromboxane receptor is blocked. Aspirin® proved to be efficient in primary and secondary prevention of ischemic events. A reduction of up to 20% of such events in high-risk patients compared to placebo was observed in large meta-analyses.[103-105]

$P2Y_{12}$ receptor antagonists are mainly used in conjunction with Aspirin ®, a combination also referred to as dual antiplatelet therapy (DAPT),[106] in patients with acute coronary syndrome and after percutaneous coronary intervention (PCI) to prevent in-stent thrombosis.[107]

$P2Y_{12}$ receptor antagonists inhibit ADP-mediated platelet activation. $P2Y_{12}$ receptor antagonists are comprised of thienopyridines (ticlopidine, clopidogrel and prasugrel) and nucleoside-nucleotide derivatives (ticagrelor, cangrelor). Thienopyridines are prodrugs that rely on conversion to active metabolites by the hepatic cytochrome P450 (CYP P450). Clopidogrel is characterized by a delayed onset of action and exhibits significant interpersonal variability of pharmacodynamics und –kinetics. In some patients, clopidogrel does not have a reliable antithrombotic effect, also referred to as high “on-treatment platelet reactivity”.[108-112] Ticagrelor - due to greater bioavailability and less response variability[113]- and prasugrel[114] have been proven to be superior to clopidogrel in patients with acute coronary syndrome.[115] In a recent study, however, the rate for primary endpoint, defined as a combination of the events, death, myocardial infarction, or stroke, was reduced by 36% under prasugrel in comparison to ticagrelor treatment.[116] Therefore, an amendment of the guidelines for acute coronary syndrome is currently under debate.[117]

Glycoprotein IIb/IIIa receptor antagonists inhibit binding of fibrinogen to activated platelets, resulting in impaired aggregate formation. Approved agents are abciximab,

tirofiban, and eptifibatide. Glycoprotein IIb/IIIa receptor antagonists are associated with an increased bleeding risk and are indicated for high-risk patients with acute coronary syndrome (ACS) undergoing or following PCI with serious risk for thrombosis or ASA/ $P2Y_{12}$ receptor antagonists- intolerance.[107]

Currently available antiplatelet drugs inhibit mechanisms pivotal for both, hemostasis and thrombosis. All agents mentioned above, have been associated with bleeding, adverse reactions[118] and mortality.[119] Up to this day, bleeding forms a serious limitation of current therapeutic approaches. Unfortunately, one principle is applicable to all agents mentioned above- the more effective the antithrombotic effect, the higher the risk of impaired hemostasis, resulting in increased hemorrhagic events.

Thus, there is the need for the evolution of more targeted therapeutic options with powerful antithrombotic effect and reduced hemorrhagic risk. New antiplatelet approaches, such as inhibitors of protein disulfide-isomerase (PDI), phosphatidylinositol 3-kinase-β (PI3Kβ), activated GPIIb/IIIa and GPVI, are currently under investigation either in preclinical or early-phase clinical trials.[112,120]

Inhibition of GPVI in platelet interaction with collagen, fibrin, and fibrinogen was investigated in this dissertation. Under normal rheological conditions, GPVI plays a minor role in hemostasis, while GPVI signaling is pivotal in atherothrombosis, making it a promising novel target for anti-atherothrombotic therapy[121] (see *GPVI as novel antiplatelet target*).

1.4 Glycoprotein VI (GPVI)

1.4.1 GPVI- structure and signal transduction

Platelet GPVI, a 60-65 kDa type I transmembrane glycoprotein and member of the immunoglobulin (Ig) superfamily, is the collagen receptor essential for collagen-mediated platelet activation, adhesion, thrombus formation, growth, and stability[122] and plays a pivotal role in maintaining vascular integrity.[123] GPVI is solely expressed on platelets and megakaryocytes.[124-126]

On the surface of resting platelets, GPVI is present as a monomer as well as a dimer and upon platelet stimulation dimerization increases.[127-129] Dimeric glycoprotein VI (GPVI) binds with much higher affinity to glycine-proline-hydroxyproline (GPO) sequence repeats in collagen fibers (KD for collagen type I and III, 42 nM and 58 nM, respectively) than monomeric GPVI (KD for collagen type I and III, 8 µM and 14 µM, respectively)[127].

Besides collagen, binding of laminin[130], fibronectin[131], vitronectin,[132] and adiponectin[133] to GPVI has been demonstrated.[130-132,134] Non-physiologic ligands are rattlesnake venom toxin, alborhagin,[135] and triple-helical collagen-related peptide (CRP), containing glycine-proline-hydroxyproline (GPO) repeat motif.[136] Notably, GPVI has shown to be activated by a group of ligands with little structural similarity, including large polysaccharides (fucoidan, dextran sulfate), diesel exhaust particles (DEP) and small peptides like LSARLAF (Leu-Ser-Ala-Arg-Leu-Ala-Phe) and histones.[137]

The transmembrane part of GPVI is non-covalently bound to the fragment crystallizable receptor γ-chain (FcRγ), which serves as signaling subunit. The FcRγ-homodimer comprises two covalently linked FcRγ-chains. The Src family kinases Fyn and Lyn[138] are associated with the FcRγ-chain. Upon GPVI activation, the kinases initiate tyrosine phosphorylation of the immunoreceptor tyrosine-based activation motif (ITAM) of the FcRγ-chain.[139,140] Subsequently, they bind to the tandem SH2 domains (“Src 2 homology domain”) of the tyrosine kinase Syk, leading to its activation.

Downstream adapters and signaling enzymes, such as LAT (linker of activated T cells), SLP76 (lymphocyte cytosolic protein 2),[141] PI3-kinase (phosphoinositide 3-kinase), Btk (Bruton’s tyrosine kinase) are set in motion, leading to the activation of the main effector enzyme PLCγ2 (phospholipase Cγ2).[142] This leads to protein kinase C activation and mobilization of intracellular Ca2+ stores, resulting in the secretion of ADP from dense granules and thromboxane formation from arachidonic acid, activation of integrin αIIbβ3 and shape change of platelets.

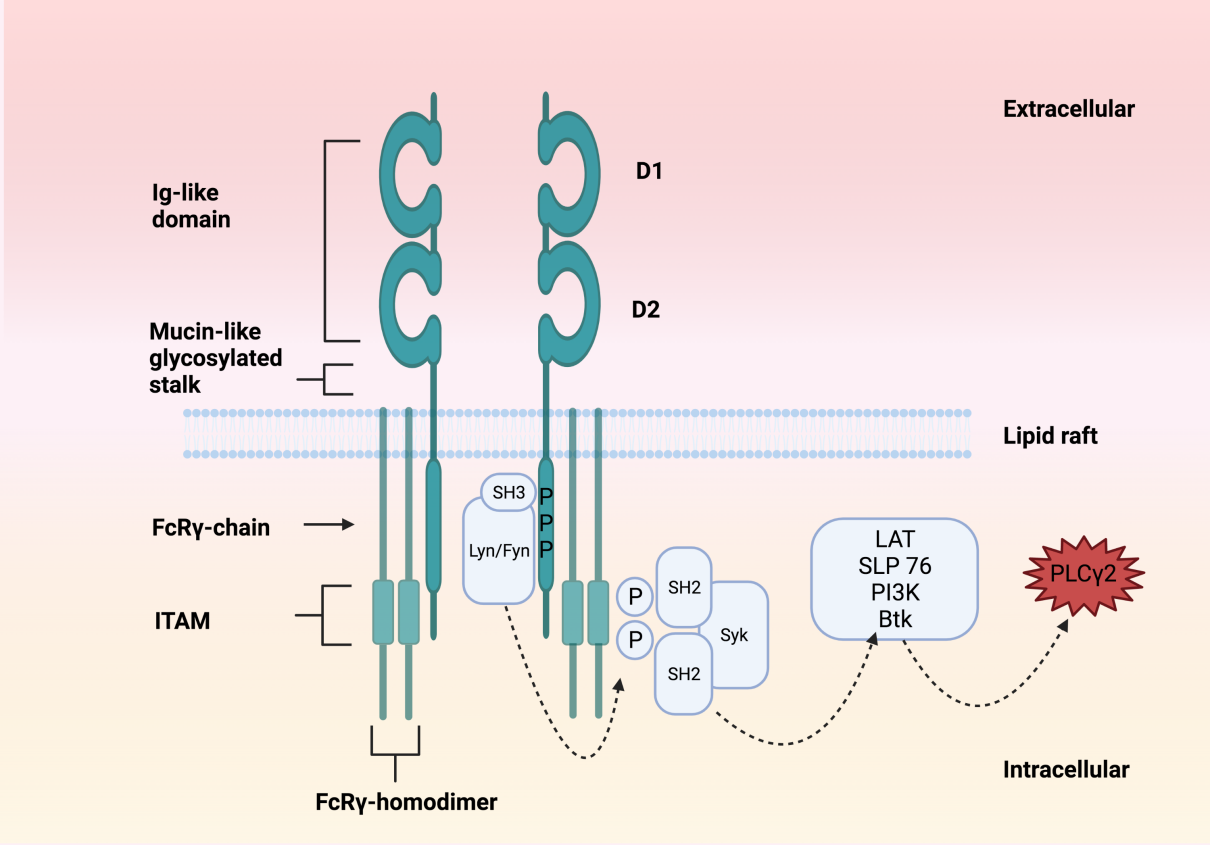

Figure 4: Structural features of the GPVI dimer and GPVI-mediated signal transduction FcRγ-chain= Fc-receptor-γ-chain, Ig= immunoglobulin; ITAM= immunoreceptor tyrosine-based activation motif, PPP= proline-rich region; Lyn/Fyn= Syk family kinases Lyn/Fyn; Syk= spleen tyrosine kinase with 2 Src-homology domains, LAT= linker of activated T cells, SLP76= lymphocyte cytosolic protein, PI3K= phosphoinositide 3-kinase, Btk= Bruton's tyrosine kinase, PLCγ_2= phospholipase Cγ_2. *Created with BioRender.com.*

1.4.2 GPVI and atherothrombosis

In healthy blood vessels, platelet adhesion and aggregation onto collagen are mediated by the two central collagen receptors- integrin α2β1 and GPVI. Platelets use both receptors to achieve stable platelet adhesion upon interaction with collagen of injured healthy arteries under arterial flow.

Atherothrombosis- arterial thrombosis driven by human atherosclerotic plaque- however, seems to be solely sustained by GPVI.[143-145] Collagen in atherosclerotic plaques generates significantly higher thrombus burden compared to collagen in healthy blood vessels. Interaction of collagen and platelets could be influenced by structural differences of the collagen fibers.[90] As mentioned before, collagen in atherosclerotic plaques is cleaved by specific metalloproteinases, which are overexpressed in those lesions due to inflammation. These small and diffuse cross-linked collagen fragments might have an increased thrombogenic potential.[95]

It was demonstrated that thrombus formation on human atherosclerotic plaque mainly occurs in two steps. In a first, rapid step, platelet adhesion and aggregation onto plaque collagen are mediated by GPVI. In a second step, thrombin and fibrin formation occurs, driven by TF.[146] These findings suggest that inhibition of GPVI could be a promising therapeutic target for the prevention of atherothrombosis due to erosion or rupture of an atherosclerotic lesion.

1.4.3 GPVI in hemostasis

While GPVI plays a significant role as collagen receptor in atherothrombosis, its impact on hemostasis could be dispensable. Clinical studies revealed that patients with GPVI-deficiency commonly exhibit mild to no bleeding tendency.[147,148] Partial compensation of GPVI-lack or malfunction by the other major collagen receptor α2β1 in normal hemostasis presents one possible explanation.

GPVI-defects can be acquired, resulting from anti-GPVI autoantibody-induced shedding of extracellular domain[149-151] or internalization of GPVI[152], or hereditary due to lack of GPVI-expression or dysfunctional expression.
Several subjects with an acquired GPVI-deficiency have been specified in literature, often associated with immune thrombocytopenia, impaired collagen-induced platelet aggregation and mild bleeding diathesis.[153] Even a case of a patient with immune GPVI-deficiency and absence of bleeding under DAPT treatment has been reported.[154] While most patients' bleeding time ranged from normal to mildly prolonged, GPVI-related defects in combination with severe thrombocytopenia can bear an increased risk for severe bleeding complications.[153]
Interestingly, GPVI-related defects occur predominantly (90%) in women and are often associated with other autoimmune disorders, including systemic lupus erythematosus,[155] Sjogren's syndrome,[156] and autoimmune thyroid disease.[157]

Congenital GPVI deficiency has not yet been researched thoroughly. Today only three reports of patients with inherited GPVI-deficiency exist. As in patients with acquired GPVI-deficiency, affected individuals presented with minor to moderate bleeding tendency.[158]
In Chile three families with congenital GPVI-deficiency due to a homozygous (hom) 2 bp insertion within the GP6 gene have been identified.[159] GP6hom platelets lack the full protein, while a normal platelet count is maintained. The GP6hom platelets showed abolished spreading and aggregate formation onto collagen and non-collagen surfaces (VWF, laminin, and rhodocytin) and impaired exposure of phosphatidylserine (PS), partially reduced thrombin generation and serotonin secretion, whilst adhesion was unaffected. Homozygous

patients showed a mild bleeding tendency and heterozygous family members were asymptomatic.

There are estimated to be about 4000 GP6hom individuals in Chile.[160] The preservation of adhesion to collagen by integrin α2β1 may explain the mild bleeding disposition of GP6hom subjects and may possibly be causative for a significant number of unreported cases. Whether these patients benefit from antithrombotic protection remains unclear.[159]

The clinical observations of minor or no bleeding in GPVI-deficient patients are supported by many studies of GPVI-deficient mouse models. Konishi et al. showed that FcR γ-chain-deficient- platelets fail to express GPVI in mice, resulting in protection against arterial thrombosis without an increase in bleeding.[145] Also, mice platelets that were genetically FcRγ- or GPVI-deficient (Gp6−/−)[161] or temporarily GPVI-depleted by administration of JAQ1,[152,162] exhibited a loss of collagen-induced platelet response and moderate-to-strong prevention of thrombosis. An increase of bleeding time was, however, only sporadically observed.[152,163 164]

In hemostasis, inhibition of GPVI function may be largely compensated due to the highly redundant function of other integrins and platelet receptors: While GPVI is critical for aggregation and PS exposure[121,162] of platelets adhering to collagen; adhesion and shaping of small aggregates on collagen is secured by the integrin α2β1.[165] Also platelet adhesion (but not aggregate formation, see above) to the injured vascular wall mediated by receptors for VWF (GPIb-IX-V), rhodocytin (CLEC-2), and laminin (α6β1) is not impaired by the lack or inhibition of GPVI.[160] In addition, vascular wall injury leads to the exposure of negatively charged phospholipids resulting in tissue factor-induced thrombin formation. Together these mechanisms could be accountable for the comparatively mild predisposition of GP6hom subjects.[160]

While there is broad agreement that inhibition or loss of GPVI entails a moderate to normal hemostasis, mechanisms by which hemostasis is secured in the absence of GPVI have not been fully understood.

1.4.4 GPVI as novel anti-atherothrombotic target

Under normal rheological conditions, GPVI plays a minor role in hemostasis, while GPVI-signaling in atherothrombosis is pivotal and constitutes a promising novel objective for anti-atherothrombotic treatment.[121]

GPVI is only expressed on platelets and megakaryocytes allowing high cell specificity while diminishing potential side effects.[124-126] On the surface of resting platelets, GPVI is present as a monomer as well as a dimer, and upon platelet stimulation, dimerization increases.[127,166,167] Therefore, targeting GPVI is not only highly platelet-specific but might also preferentially inhibit platelet activation by ruptured or eroded plaques as demonstrated by static and flow trials employing human atherosclerotic plaque material.[143,144,146,168]

In several cardiovascular diseases, platelet GPVI expression is higher and relies on the GPVI-genotype. In healthy humans, about 6000-10,000 GPVI copies per cell can be found.[169,170] Higher levels of GPVI can be found in patients with stroke and transient ischemic attack.[171] In patients with large artery disease or stroke, soluble GPVI levels are significantly elevated, suggesting that platelet function is regulated by metalloproteinase-induced shedding of GPVI.[172] Obese patients also have higher levels of GPVI, which correlate with a stronger platelet response to collagen fibers and CRP.[173] Multiple studies link GPVI polymorphisms, such as GPVI T13254C[174] and GPVI 13254CC[175] genotypes, to a greater risk of acute coronary thrombosis.

In patients with coronary heart disease, expression of GPVI at the protein and mRNA stage was found to be elevated and amplified GPVI surface expression was observed in patients with acute myocardial events, suggesting a role for GPVI expression to serve as biomarker for imminent myocardial infarction.[176] Since regulation of GPVI expression underlies DNA methylation, the degree of CpG methylation in the GPVI- promoter region of the gene is under investigation as a possible future biomarker of coronary heart disease.[177]

These clinical observations further consolidate the idea that GPVI could be a promising target to prevent acute cardiovascular events. Multiple pharmacological approaches have emerged in the past years. While GPVI-antibodies act systemically by inhibiting GPVI on all circulating platelets, GPVI-Fc probably acts locally at the side of plaque rupture or erosion by shielding collagen binding sites, leaving circulating platelets unaffected.[178,179]

Revacept® is a dimeric GPVI-Fc fusion protein and acts as a “lesion-directed competitive antagonist” to platelet GPVI, shielding GPVI epitopes exposed to the bloodstream after erosion or rupture of an atherosclerotic plaque. Revacept® is being studied in a phase II trial (ISAR-PLASTER) in patients with stable coronary artery disease undergoing elective coronary artery intervention.[180] Revacept® was well tolerated: Despite co-administration with standard antiplatelet therapy, the risk of bleeding did not increase.[181] In line with these study

results, another phase II trial with patients suffering from symptomatic carotid artery stenosis showed that additional treatment with Revacept® alongside the recommended anti-thrombotic treatment did not multiply the number of bleeding incidents.[182]

1.4.5 Fibrin(ogen) as possible GPVI ligand- current state of research

GPVI has long been known as a receptor for collagen. More recently, however, further ligands have been described, including fibronectin,[131] vitronectin,[132] laminin,[130] the hormone adiponectin,[133] and the transmembrane protein emmprin.[183]
In 2015, prior to these studies, Mammodova et al.[184] and Alshehri et al.[185] first identified fibrin as a ligand for GPVI. Mammadova et al. described binding of recombinant dimeric GPVI to fibrin but not fibrinogen. Additionally, they demonstrated that GPVI binding to fibrin supports platelets spreading, thrombin generation and tyrosine phosphorylation of Syk and the FcRy-chain regardless of integrin αIIbβ3.[184] Alshehri et al. found that GPVI shedded from the surface of platelets (supposedly monomers) binds to fibrin but not fibrinogen. In mouse models fibrin stimulated spreading and procoagulant activity of platelets via GPVI.[185] Together these studies suggest that GPVI serves as an additional platelet receptor alongside integrin αIIbβ3 in fibrin-mediated thrombus growth and stabilization.[184,185] Further, fibrin binding of GPVI was unaffected by the presence of GPRP (Gly-Pro-Arg-Pro), suggesting that it is independent of polymerization.[185]

In 2017, Onselaer et al.[186] found- contrary to Mammodova et al.- that recombinant dimeric GPVI did not bind specifically to either fibrin or fibrinogen but observed binding of monomeric GPVI to fibrin with an affinity constant of 302 nM, suggesting that monomeric GPVI binds selectively to fibrin. Furthermore, the group stated that the binding site lies within the D-region of fibrinogen and D-Dimer, not in the E-region.[186] Mangin et al.[187] described binding of monomeric GPVI to fibrinogen, but not of dimeric GPVI, while confirmation of these results in mouse models failed. Direct fibrinogen/GPVI-binding was visualized by surface plasmon resonance and by intensified adhesion of fibrinogen to human GPVI-transfected RBL-2H3 cells.[187]
In contrast, Induruwa et al.'s work showed that it is the GPVI-dimer, not GPVI-monomer which recognizes fibrinogen and fibrin (through their D-domains). Their conclusions were based on studies with recombinant monomeric GPVI and dimeric GPVI-Fc fusion proteins and flow studies using the mFab-F, which inhibits dimeric GPVI on platelets. However, close inspection of the results showed that the recombinant dimeric GPVI bound only weakly to

fibrin and fibrinogen, whereas strong binding was detected with fibrinogen D-region and D-dimer.[188]

However, all the studies described above only used purified Fg or fibrin prepared from more or less purified fibrinogen. Of note, purified fibrinogen can contain fibronectin and vitronectin that have been described to bind GPVI, and/or IgG that is known to activate platelets. In none of the studies, fibrin prepared from recombinant Fg (free of contaminating plasma proteins) or physiologically formed fibrin (in plasma or blood) has been studied. Fibrin formed in plasma is known to be different from fibrin formed from purified fibrinogen. During coagulation of plasma, multiple plasma proteins associate with fibrin fibers. 18 non-covalently and 47 covalently to fibrin bound proteins (through cross-linking via FXIIIa) have been identified, among them fibronectin, vitronectin, VWF, plasminogen.[189-192] Through incorporation of these plasma proteins into endogenous fibrin, fiber network structure and fiber morphology are altered which might result in modified platelet - activating properties for GPVI.
Fibrin generation from isolated fibrinogen is another key variable for these binding studies. Preparation varies according to concentration and purity of fibrinogen, incubation time, and methods applied to deactivate thrombin.[66,193] Important differences can be found in the recombinant GPVI-fusion proteins, as some GPVI constructs were used as monomers, some as dimers, varying in the transmembrane stalk region and the linker region.[66]

Most recently, it was demonstrated that GPVI blockage resulted in disaggregation of human thrombi formed on collagen or on human atherosclerotic plaque.[194,195] GPVI antibody-induced disaggregation of thrombi could not be reproduced with thrombi from two afribrinogenemic patients implying that the interaction of GPVI and fibrinogen is pivotal.[195]
In a ferric chloride injury model, normal onset of thrombosis but delayed occlusion in GPVI-deficient mice sparked the hypothesis that thrombus stability might be secured by fibrin/GPVI-binding in situations where exposure of collagen was discreet.[195] According to another study, polymerized fibrin, but not non-polymerized fibrin or fibrinogen, triggers GPVI-shedding.[196] Furthermore, fibrin was suggested to play a pivotal role in metalloproteolytic cleavage, which results in the release of soluble GPVI (sGPVI) into plasma through elevation of ADAM 10 activity.[197] The same group reported that inclusion of polyanionic molecules impaired fibrin-mediated platelet aggregate formation, which translated into the assertion that GPVI/fibrin- interaction could rely on electrostatic charge. The authors of these studies therefore suggested that disruption of GPVI/fibrin- engagement could be possible while sparing the GPVI/collagen- interaction.[197]

Objectives and author's contribution to publication I/II

Publication I: Dimeric Glycoprotein VI Binds to Collagen but not to Fibrin

Prior to the author's studies, only two groups had reported that platelet GPVI binds to fibrin, but not to fibrinogen, and that it is involved in fibrin-induced platelet activation[184,185] (see above). Until then, GPVI was believed to bind to plaque collagen, thereby being crucial for atherothrombosis after plaque rupture and erosion[143,146,168] but dispensable for hemostasis due to compensation by major platelet collagen receptor, integrin α2ß1.[198-201]

To further understand any potential GPVI activity in platelet activation and coagulation at the site of plaque rupture, binding of two recombinant dimeric GPVI-Fc fusion proteins (GPVI-Fc1, GPVI-Fc2) onto fibrin was studied. Fibrin was formed employing purified fibrinogen (method 1) and also generated from endogenous fibrin in plasma (method 2) or generated by exposing TF to arterially flowing blood (method 3). Plasma fibrin has been shown to differ from fibrin derived from purified fibrinogen.[202,203] Furthermore, fibrin formed by human atherosclerotic plaque (method 4) after perfusion with blood was surveyed.
Binding of recombinant dimeric GPVI-fusion proteins to fibrin was examined under static and arterial flow conditions. For studies under arterial flow conditions, fibrin-coated coverslips were mounted into parallel plate flow chambers. After perfusion with blood at a shear rate of 600/s using a syringe suction pump, GPVI-Fc binding to fibrin and collagen fibers was evaluated using advanced optical imaging, including two-photon confocal laser scanning microscopy and structured illumination microscopy.[204]

The results of the conducted studies were published in the scientific paper "*Dimeric Glycoprotein VI Binds to Collagen but not to Fibrin".*

> Ebrahim M, Jamasbi J, Adler K, Megens RTA, M'Bengue Y, Blanchet X, Uhland K, Ungerer M, Brandl R, Weber C, Elia N, Lorenz R, Münch G, Siess W. **Dimeric Glycoprotein VI Binds to Collagen but Not to Fibrin.** Thromb Haemost. 2018 Feb;118(2):351-361. doi: 10.1160/TH17-04-0302. Epub 2018 Jan 29. PMID: 29378359.

The author's contribution as principal author consisted of establishing protocols for fibrin generation, planning and execution of experiments as well as assessment and presentation of results. All experiments were conducted, and the respective paragraphs were written by the author with the exception of studies involving ELISA (Fig. 2) and human atherosclerotic plaque (Fig. 5), and the paragraph "Confocal Laser Scanning Microscopy" (method). A detailed statement of the co-authors' contribution to the publication has been submitted.

Publication II: Glycoprotein VI is not a Functional Platelet Receptor for Fibrin Formed in Plasma or Blood

Whereas binding of recombinant dimeric GPVI-Fc to different fibrins was not detected in the first publication[204], in this publication, functional assays were performed to explore the role of platelet GPVI on flow-dependent thrombus formation onto immobilized fibrinogen and variable types of fibrin under arterial flow conditions.[192]

Platelet GPVI- (both monomeric and dimeric GPVI) was inhibited by pre-incubation of blood with two different anti-GPVI antibodies (5C4, 1A5). Reduction of platelet coverage (adhesion, aggregate formation) on the different fibrins was assessed by advanced optical imaging, including two-photon confocal laser scanning microscopy and structured illumination microscopy.[192]

Fibrin, similarly to the first publication[204], was generated applying different methods: Fibrin was formed from isolated fibrinogen (referred to as "pure fibrin") or generated more physiologically from endogenous fibrinogen in plasma ("plasma fibrin") or by exposing TF to flowing blood ("blood fibrin"). Additionally, recombinant fibrinogen was obtained from a group in Japan to generate "recombinant fibrin", as a source of fibrinogen free of other plasma proteins.

Differences in protein content and covalent cross-linking mediated by FXIIIa between pure fibrin(ogen), recombinant fibrin(ogen) and plasma fibrin were identified by performing silver stainings of different fibrin(ogens). Immunoblots were conducted to examine content of vitronectin, fibronectin, FXIII, VWF and the gamma-chain of fibrinogen.

Flow chamber experiments with different fibrinogens and fibrins were conducted and inhibition of platelet adhesion and aggregation after addition of anti-GPVI antibodies (5C4, 1A5) compared to controls were evaluated.

On the platelet surface, GPVI is present in monomeric and dimeric form. After publication I had shown, that dimeric GPVI does not bind to fibrin[204], binding of monomeric GPVI to fibrin was investigated. Therefore, binding of recombinant monomeric GPVI-His to purified fibrinogen, pure fibrin, and fibrin fragments DD and E from pure fibrin was assessed using ELISA.

To further understand the role of integrin αIIbβ3 in fibrin-mediated platelet adhesion and aggregate formation, flow chamber experiments using abciximab were performed. Subsequently, the number and size of platelet aggregates after GPVI-inhibition by 5C4 or abciximab versus control were measured.[192]

The results obtained from the conducted studies were published in the scientific paper "*Glycoprotein VI is not a Functional Platelet Receptor for Fibrin Formed in Plasma or Blood".*

> Zhang D, Ebrahim M, Adler K, Blanchet X, Jamasbi J, Megens RTA, Uhland K, Ungerer M, Münch G, Deckmyn H, Weber C, Elia N, Lorenz R, Siess W. **Glycoprotein VI is not a Functional Platelet Receptor for Fibrin Formed in Plasma or Blood.** Thromb Haemost. 2020 Jun;120(6):977-993. doi: 10.1055/s-0040-1710012. Epub 2020 Jun 3. PMID: 32492725.

The author's contribution as co-author consisted of transmission of established protocols for fibrin generation to be used by the principal author, and planning and execution of flow experiments as well as assessment and presentation of the results represented in Fig. 1 A/B (control, 5C4), Fig. 2A (control, 5C4), Fig. 2E, Fig. 4 A/B (control/5C4), Fig. 5 A/B (control/5C4) (graphic modified by D. Zhang), Suppl. Fig. S1, Suppl. Fig. S2 (graphic modified by D. Zhang), Suppl. Fig. S3. Planning and discussion of experiments of Suppl. Fig. S4. Furthermore, the author initiated a cooperation with Kaketsuken (Japan) and contributed to the material transfer agreement regulating the non-commercial use of recombinant fibrinogen in the conducted studies.

A detailed statement of the co-authors' contribution to the publication has been submitted.

Summary

Platelet collagen receptor glycoprotein VI (GPVI) plays a crucial role in mediating atherothrombosis leading to ischemia of vital organs (i.e. myocardial infarction, stroke). While GPVI has been known primarily as collagen receptor, recent studies led to the identification of fibrin and fibrinogen as novel GPVI ligands. Clinical observations of patients with GPVI-deficiency revealed only minor bleeding tendency which translated into the assumption that GPVI is dispensable for hemostasis.
Binding of platelet GPVI to fibrinogen and fibrin could have essential implications for GPVI-targeting antithrombotic substances such as GPVI-fusion proteins and anti-GPVI antibodies and raise new safety concerns by directly affecting the interaction between platelets and fibrinogen/fibrin.

In the first publication of this dissertation, binding of recombinant dimeric GPVI-Fc fusion proteins with Fc from either IgG1 (GPVI-Fc1, Revacept ®) or IgG2 (GPVI-Fc2) to fibrin was assessed under static and arterial flow conditions.
Fibrin was generated applying different methods: Fibrin was formed from isolated fibrinogen (referred to as “pure fibrin”), or generated more physiologically from endogenous fibrinogen in plasma (“plasma fibrin”) or by exposing TF to flowing blood (“blood fibrin”). Fibrin generated in plasma and blood binds during the complex process of fibrin polymerization, -branching and cross-linking various plasma proteins and thus differs from fibrin prepared from purified fibrinogen. Advanced optical imaging revealed that dimeric GPVI-Fc fusion proteins bound to collagen fibers but neither to fibrin prepared from purified fibrinogen obtained from three different manufacturers, nor to “plasma fibrin” or “blood fibrin” performed by perfusion of blood over immobilized tissue factor or human atherosclerotic plaque.

In the second publication, functional *in vitro* studies under arterial flow conditions were conducted, studying the effect of anti-GPVI antibodies on fibrinogen- and fibrin-mediated platelet adhesion and aggregate formation. Additionally, recombinant fibrinogen was obtained to generate “recombinant fibrin”, as a source of fibrinogen free of other plasma proteins.
On purified fibrinogen from two different suppliers, GPVI-inhibition did not impair platelet adhesion or aggregate formation. However, inhibition of GPVI reduced platelet aggregate formation, if fibrin was prepared from purified as well as recombinant fibrinogen. However, no significant inhibition of platelet coverage with anti-GPVI antibodies was detected on more physiologically generated “plasma fibrin” or “blood fibrin”.

The discrepancy is likely to be due to the incorporation of plasma proteins into fibrin during its polymerisation, branching and cross-linking in plasma or blood. This possibly shields epitopes (such as D-regions) recognized by platelet GPVI. Thus, GPVI is not involved in platelet interaction with plasma and blood fibrin. This is relevant, since the scientific literature showing a role of GPVI for platelet interaction with “pure” fibrin and even fibrinogen is continuously increasing (see 3.4.5. *Fibrin(ogen) as possible GPVI-ligand-current state of research*).

Zusammenfassung

Der thrombozytäre Kollagen-Rezeptor GPVI spielt eine entscheidende Rolle bei der Entstehung von Atherothrombose als Ursache für Myokardinfarkte und Schlaganfälle. Bislang war GPVI als zentraler Kollagen-Rezeptor bekannt. In neueren Studien wurden Fibrin und Fibrinogen als GPVI-Liganden identifiziert. Zuvor hatte die Beobachtung, dass PatientInnen mit GPVI-Defizienz kein oder lediglich ein gering-gradig erhöhtes Blutungsrisiko zeigten, die Vorstellung geprägt, dass eine Hemmung von GPVI keinen Einfluss auf die Hämostase hat. Sollte GPVI, so wie in ersten experimentellen Studien gezeigt[184,185], an der Interaktion von Thrombozyten mit Fibrin beteiligt sein, könnte dies schwerwiegende Sicherheitsbedenken (Thrombusinstabilität, Emboliegefahr) bei dem Einsatz von GPVI-Fusionsproteinen und anti-GPVI Antikörpern zur Folge haben.

In der ersten Publikation dieser Arbeit wurden Bindungsstudien unter statischen und Flussbedingungen durchgeführt und die Bindung von GPVI-Fc Fusionsproteinen (GPVI-Fc1 und GPVI-Fc2) an Fibrin untersucht.

Fibrin wurde mittels drei unterschiedlicher Methoden generiert: Fibrin wurde aus isoliertem Fibrinogen von drei unterschiedlichen Herstellern gewonnen und als „pure fibrin“ bezeichnet. In einem zweiten Ansatz zur Simulation physiologischerer Bedingungen wurde endogenes Fibrin „plasma fibrin“ und durch Exposition von immobilisiertem Tissue Faktor gegenüber fließendem Blut „blood fibrin“ generiert. Fibrin, das in Plasma durch Aktivierung der Gerinnungskaskade entsteht, bindet zahlreiche Plasmaproteine und unterscheidet sich damit von Fibrin, das aus isoliertem Fibrinogen hergestellt wurde. Mittels hochauflösender mikroskopischer Bildgebung konnte gezeigt werden, dass dimere GPVI-Fc Fusionsproteine an Kollagen, aber weder an Fibrin aus isoliertem Fibrinogen noch physiologisch hergestelltes Fibrin binden.

In der zweiten Publikation wurden funktionelle Bindungsstudien unter arteriellen Flussbedingungen *in vitro* durchgeführt. Der Effekt einer GPVI-Inhibition durch anti-GPVI Antikörper auf Fibrinogen - und Fibrin-induzierte Thrombozytenadhäsion und –aggregation wurde untersucht. Zusätzlich wurde Fibrin aus rekombinant hergestelltem Fibrinogen generiert, um ein Plasmaprotein-freies Fibrin zu untersuchen. Eine signifikante Reduktion von Adhäsion und Aggregation konnte lediglich in den Versuchen mit Fibrin aus isoliertem oder rekombinantem Fibrinogen beobachtet werden; physiologischeres Fibrin („blood/ plasma fibrin“) zeigte keine Reduktion der Thrombozytenaggregatbildung. Auch auf isoliertem Fibrinogen ließ sich keine Hemmung der Thrombozytenadhäsion oder -aggregation durch GPVI-Inhibition zeigen. Die Diskrepanz der Befunde könnte durch Inkorporation von Plasmaproteinen in die Fibrinfasern bei der Bildung von endogenem Fibrin

und damit Maskierung von Epitopen, die für die Erkennung durch thrombozytäres GPVI wichtig sind, verursacht sein. Die Studie zeigt, dass GPVI für die Interaktion von Thrombozyten mit physiologisch gebildetem Fibrin irrelevant ist.

Paper I

Ebrahim M, Jamasbi J, Adler K, Megens RTA, M'Bengue Y, Blanchet X, Uhland K, Ungerer M, Brandl R, Weber C, Elia N, Lorenz R, Münch G, Siess W. **Dimeric Glycoprotein VI Binds to Collagen but Not to Fibrin.** Thromb Haemost. 2018 Feb;118(2):351-361. doi: 10.1160/TH17-04-0302. Epub 2018 Jan 29. PMID: 29378359.

Paper II

Zhang D, Ebrahim M, Adler K, Blanchet X, Jamasbi J, Megens RTA, Uhland K, Ungerer M, Münch G, Deckmyn H, Weber C, Elia N, Lorenz R, Siess W. **Glycoprotein VI is not a Functional Platelet Receptor for Fibrin Formed in Plasma or Blood.** Thromb Haemost. 2020 Jun;120(6):977-993. doi: 10.1055/s-0040-1710012. Epub 2020 Jun 3. PMID: 32492725.

Bibliography

1. Schimmelbusch C. Die Blutplättchen und die Blutgerinnung. *Archiv für pathologische Anatomie und Physiologie und für klinische Medicin.* 1885(101):201-244.

2. O'Brien JR. The adhesiveness of native platelets and its prevention. *J Clin Pathol.* Mar 1961;14:140-149.

3. Bizzozero J. Ueber einen neuen Formbestandteil des Blutes und dessen Rolle bei der Thrombose und Blutgerinnung. *Archiv für pathologische Anatomie und Physiologie und für klinische Medizin.* 1882;90:261-332.

4. BioRender.com. Modified and adapted from “Blood Clot formation in broken vessel”, by BioRender.com (2021). Retrieved from https://app.biorender.com/biorender-templates.

5. Behnke O, Forer A. From megakaryocytes to platelets: platelet morphogenesis takes place in the bloodstream. *Eur J Haematol Suppl.* 1998;61:3-23.

6. Booyse FM, Zschocke D, Hoveke TP, Rafelson ME, Jr. Studies on human platelets. IV. Protein synthesis in maturing human platelets. *Thromb Diath Haemorrh.* Aug 31 1971;26(1):167-176.

7. Born GV, Dearnley R, Foulks JG, Sharp DE. Quantification of the morphological reaction of platelets to aggregating agents and of its reversal by aggregation inhibitors. *J Physiol.* Jul 1978;280:193-212.

8. Hattori A. SM. A study on platelet shape change and its relation to function. *Blood Vessel.* 1977(8):588-594.

9. Morgenstern E. Platelets morphology/ultrastructure. In: Bruchhausen Fv., Walter U (edits.) Platelets and their factors. . *Handbook of experimental pharmacology. Springer Verlag, Heidelberg* 1997:27-52.

10. Eberth JC, Schimmelbusch, C. Experimentelle Untersuchung über Thrombose. *Archiv für pathologische Anatomie und Physiologie und für klinische Medizin.* 1886(103):39-87.

11. Polanowska-Grabowska R, Simon CG, Jr., Gear AR. Platelet adhesion to collagen type I, collagen type IV, von Willebrand factor, fibronectin, laminin and fibrinogen: rapid kinetics under shear. *Thromb Haemost.* Jan 1999;81(1):118-123.

12. Kroll MH, Harris TS, Moake JL, Handin RI, Schafer AI. von Willebrand factor binding to platelet GpIb initiates signals for platelet activation. *J Clin Invest.* Nov 1991;88(5):1568-1573.

13. Berndt MC, Du XP, Booth WJ. Ristocetin-dependent reconstitution of binding of von Willebrand factor to purified human platelet membrane glycoprotein Ib-IX complex. *Biochemistry.* Jan 26 1988;27(2):633-640.

14. Chow TW, Hellums JD, Moake JL, Kroll MH. Shear stress-induced von Willebrand factor binding to platelet glycoprotein Ib initiates calcium influx associated with aggregation. *Blood.* Jul 1 1992;80(1):113-120.

15. Ikeda Y, Handa M, Kamata T, et al. Transmembrane calcium influx associated with von Willebrand factor binding to GP Ib in the initiation of shear-induced platelet aggregation. *Thromb Haemost.* May 3 1993;69(5):496-502.

16. Lopez JA. The platelet glycoprotein Ib-IX complex. *Blood Coagul Fibrinolysis.* Feb 1994;5(1):97-119.

17. Doggett TA, Girdhar G, Lawshe A, et al. Selectin-like kinetics and biomechanics promote rapid platelet adhesion in flow: the GPIb(alpha)-vWF tether bond. *Biophys J.* Jul 2002;83(1):194-205.

18. Roth GJ, Titani K, Hoyer LW, Hickey MJ. Localization of binding sites within human von Willebrand factor for monomeric type III collagen. *Biochemistry.* Dec 30 1986;25(26):8357-8361.

19. Matsushita T, Meyer D, Sadler JE. Localization of von willebrand factor-binding sites for platelet glycoprotein Ib and botrocetin by charged-to-alanine scanning mutagenesis. *J Biol Chem.* Apr 14 2000;275(15):11044-11049.

20. Shimizu A, Matsushita T, Kondo T, et al. Identification of the amino acid residues of the platelet glycoprotein Ib (GPIb) essential for the von Willebrand factor binding by clustered charged-to-alanine scanning mutagenesis. *J Biol Chem.* Apr 16 2004;279(16):16285-16294.

21. Kasirer-Friede A, Moran B, Nagrampa-Orje J, et al. ADAP is required for normal alphaIIbbeta3 activation by VWF/GP Ib-IX-V and other agonists. *Blood.* Feb 1 2007;109(3):1018-1025.

22. Stel HV, Sakariassen KS, de Groot PG, van Mourik JA, Sixma JJ. Von Willebrand factor in the vessel wall mediates platelet adherence. *Blood.* Jan 1985;65(1):85-90.

23. Rabie T, Varga-Szabo D, Bender M, et al. Diverging signaling events control the pathway of GPVI down-regulation in vivo. *Blood.* Jul 15 2007;110(2):529-535.

24. Zheng YM, Liu C, Chen H, Locke D, Ryan JC, Kahn ML. Expression of the platelet receptor GPVI confers signaling via the Fc receptor gamma -chain in response to the snake venom convulxin but not to collagen. *J Biol Chem.* Apr 20 2001;276(16):12999-13006.

25. Moroi M, Jung SM. Integrin-mediated platelet adhesion. *Front Biosci.* Jul 23 1998;3:d719-728.

26. Varga-Szabo D, Pleines I, Nieswandt B. Cell adhesion mechanisms in platelets. *Arterioscler Thromb Vasc Biol.* Mar 2008;28(3):403-412.

27. Born GV, Kratzer MA. Source and concentration of extracellular adenosine triphosphate during haemostasis in rats, rabbits and man. *J Physiol.* Sep 1984;354:419-429.

28. Born GV, Feinberg H. Binding of adenosine diphosphate to intact human platelets. *J Physiol.* Oct 1975;251(3):803-816.

29. Offermanns S, Toombs CF, Hu YH, Simon MI. Defective platelet activation in G alpha(q)-deficient mice. *Nature.* Sep 11 1997;389(6647):183-186.

30. Hollopeter G, Jantzen HM, Vincent D, et al. Identification of the platelet ADP receptor targeted by antithrombotic drugs. *Nature.* Jan 11 2001;409(6817):202-207.

31. Jin J, Quinton TM, Zhang J, Rittenhouse SE, Kunapuli SP. Adenosine diphosphate (ADP)-induced thromboxane A(2) generation in human platelets requires coordinated signaling through integrin alpha(IIb)beta(3) and ADP receptors. *Blood.* Jan 1 2002;99(1):193-198.

32. Svensson J, Hamberg M, Samuelsson B. On the formation and effects of thromboxane A2 in human platelets. *Acta Physiol Scand.* Nov 1976;98(3):285-294.

33. Budnik I, Shenkman B, Savion N. Synergistic effect of signaling from receptors of soluble platelet agonists and outside-in signaling in formation of a stable fibrinogen-integrin alphaIIbbeta3-actin cytoskeleton complex. *Thromb Res.* Jan 2015;135(1):114-120.

34. Glanzmann E. Commentary on and reprint of Glanzmann E, Hereditäre häemorrhagische thrombasthenie. Ein Beitrag zur Pathologie der Blutplättchen [Hereditary hemorrhagic thrombasthenia: A contribution on the pathology of blood platelets], in Jahrbuch für Kinderheilkunde (1918) 88:113–141. *Hematology.* 2000(I-III):55-94.

35. Baumgartner HR, Muggli R, Tschopp TB, Turitto VT. Platelet adhesion, release and aggregation in flowing blood: effects of surface properties and platelet function. *Thromb Haemost.* Feb 29 1976;35(1):124-138.

36. Wilcox JN, Smith KM, Schwartz SM, Gordon D. Localization of tissue factor in the normal vessel wall and in the atherosclerotic plaque. *Proc Natl Acad Sci U S A.* Apr 1989;86(8):2839-2843.

37. Bevilacqua MP, Pober JS, Majeau GR, Cotran RS, Gimbrone MA, Jr. Interleukin 1 (IL-1) induces biosynthesis and cell surface expression of procoagulant activity in human vascular endothelial cells. *J Exp Med.* Aug 1 1984;160(2):618-623.

38. Semeraro N, Biondi A, Lorenzet R, Locati D, Mantovani A, Donati MB. Direct induction of tissue factor synthesis by endotoxin in human macrophages from diverse anatomical sites. *Immunology.* Dec 1983;50(4):529-535.

39. Panes O, Matus V, Saez CG, Quiroga T, Pereira J, Mezzano D. Human platelets synthesize and express functional tissue factor. *Blood.* Jun 15 2007;109(12):5242-5250.

40. Dubois C, Panicot-Dubois L, Gainor JF, Furie BC, Furie B. Thrombin-initiated platelet activation in vivo is vWF independent during thrombus formation in a laser injury model. *J Clin Invest.* Apr 2007;117(4):953-960.

41. Dubois C, Panicot-Dubois L, Merrill-Skoloff G, Furie B, Furie BC. Glycoprotein VI-dependent and -independent pathways of thrombus formation in vivo. *Blood.* May 15 2006;107(10):3902-3906.

42. BioRender.com. Modified and adapted from “Platelet activation”, by BioRender.com (2021). Retrieved from https://app.biorender.com/biorender-templates.

43. Jamasbi J. *Vergleich bekannter und Entwicklung neuer therapeutischer Ansätze zur Hemmung der Interaktion von thrombozytärem Glykoprotein VI mit atherosklerotischem Plaque. Dissertation, LMU München: Medizinische Fakultät*2017.

44. Davie EW, Ratnoff OD. Waterfall Sequence for Intrinsic Blood Clotting. *Science.* Sep 18 1964;145(3638):1310-1312.

45. Mackman N, Tilley RE, Key NS. Role of the extrinsic pathway of blood coagulation in hemostasis and thrombosis. *Arterioscler Thromb Vasc Biol.* Aug 2007;27(8):1687-1693.

46. Nossel HL. Differential consumption of coagulation factors resulting from activation of the extrinsic (tissue thromboplastin) or the intrinsic (foreign surface contact) pathways. *Blood.* Mar 1967;29(3):331-340.

47. Macfarlane RG. An Enzyme Cascade in the Blood Clotting Mechanism, and Its Function as a Biochemical Amplifier. *Nature.* May 2 1964;202:498-499.

48. Wikipedia.com. Coagulation; 2020. https://en.wikipedia.org/wiki/Coagulation. . Accessed February 17, 2020.

49. Davie EW, Fujikawa K, Kisiel W. The coagulation cascade: initiation, maintenance, and regulation. *Biochemistry.* Oct 29 1991;30(43):10363-10370.

50. Maas C, Renne T. Coagulation factor XII in thrombosis and inflammation. *Blood.* Apr 26 2018;131(17):1903-1909.

51. Mezger M, Nording H, Sauter R, et al. Platelets and Immune Responses During Thromboinflammation. *Front Immunol.* 2019;10:1731.

52. Macfarlane RG. The Activation and Consumption of Factor X in Recalcified Plasma. *British Journal of Haematology.* 1964;10(2):217-224.

53. Margolis J, Bishop EA. Studies on Plasma Kinins. I. The Composition of Kininogen Complex. *Aust J Exp Biol Med Sci.* Aug 1963;41:293-306.

54. Hardisty RM, Margolis J. The role of Hageman factor in the initiation of blood coagulation. *Br J Haematol.* Apr 1959;5(2):203-211.

55. Ratnoff OD, Rosenblum JM. Role of Hageman factor in the initiation of clotting by glass; evidence that glass frees Hageman factor from inhibition. *Am J Med.* Aug 1958;25(2):160-168.

56. Ratnoff OD, Davie EW, Mallett DL. Studies on the action of Hageman factor: evidence that activated Hageman factor in turn activates plasma thromboplastin antecedent. *J Clin Invest.* May 1961;40:803-819.

57. Ratnoff OD, Davie E.W. The Activation of Christmas Factor (Factor IX) by Activated Plasma Thromboplastin Antecedent (Activated Factor XI). *Biochemistry.* 1962;1,4:677-685.

58. Margolis J. Glass surface and blood coagulation. *Nature.* Oct 13 1956;178(4537):805-806.

59. Margolis J. The interrelationship of coagulation of plasma and release of peptides. *Ann N Y Acad Sci.* Feb 4 1963;104:133-145.

60. BioRender.com. Modified and adapted from “Coagulation Cascade”, by BioRender.com (2021). Retrieved from https://app.biorender.com/biorender-templates. . 2021.

61. Henschen A, Lottspeich F, Kehl M, Southan C. Covalent structure of fibrinogen. *Ann N Y Acad Sci.* Jun 27 1983;408:28-43.

62. Collen D, Kudryk B, Hessel B, Blomback B. Primary structure of human fibrinogen and fibrin. Isolation and partial characterization of chains of fragment D. *J Biol Chem.* Aug 10 1975;250(15):5808-5817.

63. Blomback B, Hessel B, Hogg D, Therkildsen L. A two-step fibrinogen--fibrin transition in blood coagulation. *Nature.* Oct 12 1978;275(5680):501-505.

64. Mosesson MW. Fibrinogen and fibrin structure and functions. *J Thromb Haemost.* Aug 2005;3(8):1894-1904.

65. Macfarlane RG. The Development of Ideas on Fibrinolysis. *Br Med Bull.* Sep 1964;20:173-178.

66. Slater A, Perrella G, Onselaer MB, et al. Does fibrin(ogen) bind to monomeric or dimeric GPVI, or not at all? *Platelets.* 2019;30(3):281-289.

67. Schwenke DC, Carew TE. Initiation of atherosclerotic lesions in cholesterol-fed rabbits. I. Focal increases in arterial LDL concentration precede development of fatty streak lesions. *Arteriosclerosis.* Nov-Dec 1989;9(6):895-907.

68. Falk E. Unstable angina with fatal outcome: dynamic coronary thrombosis leading to infarction and/or sudden death. Autopsy evidence of recurrent mural thrombosis with peripheral embolization culminating in total vascular occlusion. *Circulation.* Apr 1985;71(4):699-708.

69. Davies MJ, Bland JM, Hangartner JR, Angelini A, Thomas AC. Factors influencing the presence or absence of acute coronary artery thrombi in sudden ischaemic death. *Eur Heart J.* Mar 1989;10(3):203-208.

70. Collaborators GBDCoD. Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet.* Nov 10 2018;392(10159):1736-1788.

71. Yusuf S, Hawken S, Ounpuu S, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. *Lancet.* Sep 11-17 2004;364(9438):937-952.

72. Roth GA, Johnson C, Abajobir A, et al. Global, Regional, and National Burden of Cardiovascular Diseases for 10 Causes, 1990 to 2015. *J Am Coll Cardiol.* Jul 4 2017;70(1):1-25.

73. Ross R. The pathogenesis of atherosclerosis--an update. *N Engl J Med.* Feb 20 1986;314(8):488-500.

74. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature.* Apr 29 1993;362(6423):801-809.

75. Libby P. Inflammation in atherosclerosis. *Nature.* Dec 19-26 2002;420(6917):868-874.

76. Mahalingam A, Gawandalkar UU, Kini G, et al. Numerical analysis of the effect of turbulence transition on the hemodynamic parameters in human coronary arteries. *Cardiovasc Diagn Ther.* Jun 2016;6(3):208-220.

77. Egashira K, Zhao Q, Kataoka C, et al. Importance of monocyte chemoattractant protein-1 pathway in neointimal hyperplasia after periarterial injury in mice and monkeys. *Circ Res.* Jun 14 2002;90(11):1167-1172.

78. Gu L, Okada Y, Clinton SK, et al. Absence of monocyte chemoattractant protein-1 reduces atherosclerosis in low density lipoprotein receptor-deficient mice. *Mol Cell.* Aug 1998;2(2):275-281.

79. Halden Y, Rek A, Atzenhofer W, Szilak L, Wabnig A, Kungl AJ. Interleukin-8 binds to syndecan-2 on human endothelial cells. *Biochem J.* Jan 15 2004;377(Pt 2):533-538.

80. Collins RG, Velji R, Guevara NV, Hicks MJ, Chan L, Beaudet AL. P-Selectin or intercellular adhesion molecule (ICAM)-1 deficiency substantially protects against atherosclerosis in apolipoprotein E-deficient mice. *J Exp Med.* Jan 3 2000;191(1):189-194.

81. Henn V, Slupsky JR, Grafe M, et al. CD40 ligand on activated platelets triggers an inflammatory reaction of endothelial cells. *Nature.* Feb 5 1998;391(6667):591-594.

82. Johnson RC, Chapman SM, Dong ZM, et al. Absence of P-selectin delays fatty streak formation in mice. *J Clin Invest.* Mar 1 1997;99(5):1037-1043.

83. Navab M, Imes SS, Hama SY, et al. Monocyte transmigration induced by modification of low density lipoprotein in cocultures of human aortic wall cells is due to induction of monocyte chemotactic protein 1 synthesis and is abolished by high density lipoprotein. *J Clin Invest.* Dec 1991;88(6):2039-2046.

84. Palinski W, Rosenfeld ME, Yla-Herttuala S, et al. Low density lipoprotein undergoes oxidative modification in vivo. *Proc Natl Acad Sci U S A.* Feb 1989;86(4):1372-1376.

85. Sparrow CP, Parthasarathy S, Steinberg D. A macrophage receptor that recognizes oxidized low density lipoprotein but not acetylated low density lipoprotein. *J Biol Chem.* Feb 15 1989;264(5):2599-2604.

86. Feil S, Fehrenbacher B, Lukowski R, et al. Transdifferentiation of vascular smooth muscle cells to macrophage-like cells during atherogenesis. *Circ Res.* Sep 12 2014;115(7):662-667.

87. Bigg HF, Rowan AD, Barker MD, Cawston TE. Activity of matrix metalloproteinase-9 against native collagen types I and III. *FEBS J.* Mar 2007;274(5):1246-1255.

88. Sukhova GK, Schonbeck U, Rabkin E, et al. Evidence for increased collagenolysis by interstitial collagenases-1 and -3 in vulnerable human atheromatous plaques. *Circulation.* May 18 1999;99(19):2503-2509.

89. Deguchi JO, Aikawa E, Libby P, et al. Matrix metalloproteinase-13/collagenase-3 deletion promotes collagen accumulation and organization in mouse atherosclerotic plaques. *Circulation.* Oct 25 2005;112(17):2708-2715.

90. Guglielmini G, Appolloni V, Momi S, et al. Matrix metalloproteinase-2 enhances platelet deposition on collagen under flow conditions. *Thromb Haemost.* Jan 2016;115(2):333-343.

91. Lenti M, Falcinelli E, Pompili M, et al. Matrix metalloproteinase-2 of human carotid atherosclerotic plaques promotes platelet activation. Correlation with ischaemic events. *Thromb Haemost.* Jun 2014;111(6):1089-1101.

92. Bentzon JF, Otsuka F, Virmani R, Falk E. Mechanisms of plaque formation and rupture. *Circ Res.* Jun 6 2014;114(12):1852-1866.

93. Davies MJ, Thomas A. Thrombosis and acute coronary-artery lesions in sudden cardiac ischemic death. *N Engl J Med.* May 3 1984;310(18):1137-1140.

94. Falk E. Plaque rupture with severe pre-existing stenosis precipitating coronary thrombosis. Characteristics of coronary atherosclerotic plaques underlying fatal occlusive thrombi. *Br Heart J.* Aug 1983;50(2):127-134.

95. Newby AC. Metalloproteinases and vulnerable atherosclerotic plaques. *Trends Cardiovasc Med.* Nov 2007;17(8):253-258.

96. Fernandez-Ortiz A, Badimon JJ, Falk E, et al. Characterization of the relative thrombogenicity of atherosclerotic plaque components: implications for consequences of plaque rupture. *J Am Coll Cardiol.* Jun 1994;23(7):1562-1569.

97. Stone GW, Maehara A, Mintz GS. The reality of vulnerable plaque detection. *JACC Cardiovasc Imaging.* Aug 2011;4(8):902-904.

98. Kolodgie FD, Burke AP, Farb A, et al. Differential accumulation of proteoglycans and hyaluronan in culprit lesions: insights into plaque erosion. *Arterioscler Thromb Vasc Biol.* Oct 1 2002;22(10):1642-1648.

99. Lafont A. Basic aspects of plaque vulnerability. *Heart.* Oct 2003;89(10):1262-1267.

100. Narula J, Nakano M, Virmani R, et al. Histopathologic characteristics of atherosclerotic coronary disease and implications of the findings for the invasive and noninvasive detection of vulnerable plaques. *J Am Coll Cardiol.* Mar 12 2013;61(10):1041-1051.

101. Spronk HMH, Padro T, Siland JE, et al. Atherothrombosis and Thromboembolism: Position Paper from the Second Maastricht Consensus Conference on Thrombosis. *Thromb Haemost.* Feb 2018;118(2):229-250.

102. Aikawa M, Rabkin E, Okada Y, et al. Lipid lowering by diet reduces matrix metalloproteinase activity and increases collagen content of rabbit atheroma: a potential mechanism of lesion stabilization. *Circulation.* Jun 23 1998;97(24):2433-2444.

103. Hart RG, Halperin JL, McBride R, Benavente O, Man-Son-Hing M, Kronmal RA. Aspirin for the primary prevention of stroke and other major vascular events: meta-analysis and hypotheses. *Arch Neurol.* Mar 2000;57(3):326-332.

104. Antithrombotic Trialists C, Baigent C, Blackwell L, et al. Aspirin in the primary and secondary prevention of vascular disease: collaborative meta-analysis of individual participant data from randomised trials. *Lancet.* May 30 2009;373(9678):1849-1860.

105. Raju N, Sobieraj-Teague M, Hirsh J, O'Donnell M, Eikelboom J. Effect of aspirin on mortality in the primary prevention of cardiovascular disease. *Am J Med.* Jul 2011;124(7):621-629.

106. Udell JA, Bonaca MP, Collet JP, et al. Long-term dual antiplatelet therapy for secondary prevention of cardiovascular events in the subgroup of patients with previous myocardial infarction: a collaborative meta-analysis of randomized trials. *Eur Heart J.* Jan 21 2016;37(4):390-399.

107. Collet JP, Thiele H, Barbato E, et al. 2020 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. *Eur Heart J.* Aug 29 2020.

108. Jaremo P, Lindahl TL, Fransson SG, Richter A. Individual variations of platelet inhibition after loading doses of clopidogrel. *J Intern Med.* Sep 2002;252(3):233-238.

109. Matetzky S, Shenkman B, Guetta V, et al. Clopidogrel resistance is associated with increased risk of recurrent atherothrombotic events in patients with acute myocardial infarction. *Circulation.* Jun 29 2004;109(25):3171-3175.

110. Muller I, Besta F, Schulz C, Massberg S, Schonig A, Gawaz M. Prevalence of clopidogrel non-responders among patients with stable angina pectoris scheduled for elective coronary stent placement. *Thromb Haemost.* May 2003;89(5):783-787.

111. Serebruany VL, Steinhubl SR, Berger PB, Malinin AI, Bhatt DL, Topol EJ. Variability in platelet responsiveness to clopidogrel among 544 individuals. *J Am Coll Cardiol.* Jan 18 2005;45(2):246-251.

112. Tscharre M, Michelson AD, Gremmel T. Novel Antiplatelet Agents in Cardiovascular Disease. *J Cardiovasc Pharmacol Ther.* May 2020;25(3):191-200.

113. van Giezen JJ, Berntsson P, Zachrisson H, Bjorkman JA. Comparison of ticagrelor and thienopyridine P2Y(12) binding characteristics and antithrombotic and bleeding effects in rat and dog models of thrombosis/hemostasis. *Thromb Res.* Nov 2009;124(5):565-571.

114. Wiviott SD, Braunwald E, McCabe CH, et al. Prasugrel versus clopidogrel in patients with acute coronary syndromes. *N Engl J Med.* Nov 15 2007;357(20):2001-2015.

115. Wallentin L, Becker RC, Budaj A, et al. Ticagrelor versus clopidogrel in patients with acute coronary syndromes. *N Engl J Med.* Sep 10 2009;361(11):1045-1057.

116. Schupke S, Neumann FJ, Menichelli M, et al. Ticagrelor or Prasugrel in Patients with Acute Coronary Syndromes. *N Engl J Med.* Oct 17 2019;381(16):1524-1534.

117. Overbeck P. Unerwarteter Sieger beim Direktvergleich: Prasugrel schlägt Ticagrelor. 2019; https://www.kardiologie.org/esc-kongress-2019/unerwarteter-sieger-beim-direktvergleich--prasugrel-schlaegt-tic/17128104. Accessed December 06,2020.

118. Gozzo L, Navarria A, Benfatto G, et al. Safety of Antiplatelet Agents: Analysis of 'Real-World' Data from the Italian National Pharmacovigilance Network. *Clin Drug Investig.* Nov 2017;37(11):1067-1081.

119. Ahmad T, Davies R, Alagona P, Jr. The Benefits and Risks of Oral Antiplatelet Therapy in Patients with Acute Coronary Syndrome. *J Fam Pract.* Feb 2017;66(2 Suppl).

120. McFadyen JD, Schaff M, Peter K. Current and future antiplatelet therapies: emphasis on preserving haemostasis. *Nat Rev Cardiol.* Mar 2018;15(3):181-191.

121. Nieswandt B, Brakebusch C, Bergmeier W, et al. Glycoprotein VI but not alpha2beta1 integrin is essential for platelet interaction with collagen. *EMBO J.* May 1 2001;20(9):2120-2130.

122. Rayes J, Watson SP, Nieswandt B. Functional significance of the platelet immune receptors GPVI and CLEC-2. *J Clin Invest.* Jan 2 2019;129(1):12-23.

123. Boulaftali Y, Mawhin MA, Jandrot-Perrus M, Ho-Tin-Noe B. Glycoprotein VI in securing vascular integrity in inflamed vessels. *Res Pract Thromb Haemost.* Apr 2018;2(2):228-239.

124. Nieswandt B, Watson SP. Platelet-collagen interaction: is GPVI the central receptor? *Blood.* Jul 15 2003;102(2):449-461.

125. Clemetson JM, Polgar J, Magnenat E, Wells TN, Clemetson KJ. The platelet collagen receptor glycoprotein VI is a member of the immunoglobulin superfamily closely related to FcalphaR and the natural killer receptors. *J Biol Chem.* Oct 8 1999;274(41):29019-29024.

126. Jandrot-Perrus M, Busfield S, Lagrue AH, et al. Cloning, characterization, and functional studies of human and mouse glycoprotein VI: a platelet-specific collagen receptor from the immunoglobulin superfamily. *Blood.* Sep 1 2000;96(5):1798-1807.

127. Jung SM, Moroi M, Soejima K, et al. Constitutive dimerization of glycoprotein VI (GPVI) in resting platelets is essential for binding to collagen and activation in flowing blood. *J Biol Chem.* Aug 24 2012;287(35):30000-30013.

128. Jung SM, Tsuji K, Moroi M. Glycoprotein (GP) VI dimer as a major collagen-binding site of native platelets: direct evidence obtained with dimeric GPVI-specific Fabs. *J Thromb Haemost.* Aug 2009;7(8):1347-1355.

129. Loyau S, Dumont B, Ollivier V, et al. Platelet glycoprotein VI dimerization, an active process inducing receptor competence, is an indicator of platelet reactivity. *Arterioscler Thromb Vasc Biol.* Mar 2012;32(3):778-785.

130. Inoue O, Suzuki-Inoue K, McCarty OJ, et al. Laminin stimulates spreading of platelets through integrin alpha6beta1-dependent activation of GPVI. *Blood.* Feb 15 2006;107(4):1405-1412.

131. Bultmann A, Li Z, Wagner S, et al. Impact of glycoprotein VI and platelet adhesion on atherosclerosis--a possible role of fibronectin. *J Mol Cell Cardiol.* Sep 2010;49(3):532-542.

132. Schonberger T, Ziegler M, Borst O, et al. The dimeric platelet collagen receptor GPVI-Fc reduces platelet adhesion to activated endothelium and preserves myocardial function after transient ischemia in mice. *Am J Physiol Cell Physiol.* Oct 1 2012;303(7):C757-766.

133. Riba R, Hughes CE, Graham A, Watson SP, Naseem KM. Globular adiponectin induces platelet activation through the collagen receptor GPVI-Fc receptor gamma chain complex. *J Thromb Haemost.* Jun 2008;6(6):1012-1020.

134. Kageyama S, Yamamoto H, Nakazawa H, Yoshimoto R. Anti-human vWF monoclonal antibody, AJvW-2 Fab, inhibits repetitive coronary artery thrombosis without bleeding time prolongation in dogs. *Thromb Res.* Mar 1 2001;101(5):395-404.

135. Andrews RK, Gardiner EE, Asazuma N, et al. A novel viper venom metalloproteinase, alborhagin, is an agonist at the platelet collagen receptor GPVI. *J Biol Chem.* Jul 27 2001;276(30):28092-28097.

136. Asselin J, Knight CG, Farndale RW, Barnes MJ, Watson SP. Monomeric (glycine-proline-hydroxyproline)10 repeat sequence is a partial agonist of the platelet collagen receptor glycoprotein VI. *Biochem J.* Apr 15 1999;339 (Pt 2):413-418.

137. Alshehri OM, Montague S, Watson S, et al. Activation of glycoprotein VI (GPVI) and C-type lectin-like receptor-2 (CLEC-2) underlies platelet activation by diesel exhaust particles and other charged/hydrophobic ligands. *Biochem J.* Jun 15 2015;468(3):459-473.

138. Ezumi Y, Shindoh K, Tsuji M, Takayama H. Physical and functional association of the Src family kinases Fyn and Lyn with the collagen receptor glycoprotein VI-Fc receptor gamma chain complex on human platelets. *J Exp Med.* Jul 20 1998;188(2):267-276.

139. Gibbins JM, Okuma M, Farndale R, Barnes M, Watson SP. Glycoprotein VI is the collagen receptor in platelets which underlies tyrosine phosphorylation of the Fc receptor gamma-chain. *FEBS Lett.* Aug 18 1997;413(2):255-259.

140. Tsuji M, Ezumi Y, Arai M, Takayama H. A novel association of Fc receptor gamma-chain with glycoprotein VI and their co-expression as a collagen receptor in human platelets. *J Biol Chem.* Sep 19 1997;272(38):23528-23531.

141. Judd BA, Myung PS, Obergfell A, et al. Differential requirement for LAT and SLP-76 in GPVI versus T cell receptor signaling. *J Exp Med.* Mar 18 2002;195(6):705-717.

142. Watson SP, Asazuma N, Atkinson B, et al. The role of ITAM- and ITIM-coupled receptors in platelet activation by collagen. *Thromb Haemost.* Jul 2001;86(1):276-288.

143. Penz S, Reininger AJ, Brandl R, et al. Human atheromatous plaques stimulate thrombus formation by activating platelet glycoprotein VI. *FASEB J.* Jun 2005;19(8):898-909.

144. Schulz C, Penz S, Hoffmann C, et al. Platelet GPVI binds to collagenous structures in the core region of human atheromatous plaque and is critical for atheroprogression in vivo. *Basic Res Cardiol.* Jul 2008;103(4):356-367.

145. Konishi H, Katoh Y, Takaya N, et al. Platelets activated by collagen through immunoreceptor tyrosine-based activation motif play pivotal role in initiation and generation of neointimal hyperplasia after vascular injury. *Circulation.* Feb 26 2002;105(8):912-916.

146. Reininger AJ, Bernlochner I, Penz SM, et al. A 2-step mechanism of arterial thrombus formation induced by human atherosclerotic plaques. *J Am Coll Cardiol.* Mar 16 2010;55(11):1147-1158.

147. Arai M, Yamamoto N, Moroi M, Akamatsu N, Fukutake K, Tanoue K. Platelets with 10% of the normal amount of glycoprotein VI have an impaired response to collagen that results in a mild bleeding tendency. *Br J Haematol.* Jan 1995;89(1):124-130.

148. Boylan B, Chen H, Rathore V, et al. Anti-GPVI-associated ITP: an acquired platelet disorder caused by autoantibody-mediated clearance of the GPVI/FcRgamma-chain complex from the human platelet surface. *Blood.* Sep 1 2004;104(5):1350-1355.

149. Stephens G, Yan Y, Jandrot-Perrus M, Villeval JL, Clemetson KJ, Phillips DR. Platelet activation induces metalloproteinase-dependent GP VI cleavage to down-regulate platelet reactivity to collagen. *Blood.* Jan 1 2005;105(1):186-191.

150. Bergmeier W, Rabie T, Strehl A, et al. GPVI down-regulation in murine platelets through metalloproteinase-dependent shedding. *Thromb Haemost.* May 2004;91(5):951-958.

151. Gardiner EE, Arthur JF, Kahn ML, Berndt MC, Andrews RK. Regulation of platelet membrane levels of glycoprotein VI by a platelet-derived metalloproteinase. *Blood.* Dec 1 2004;104(12):3611-3617.

152. Nieswandt B, Schulte V, Bergmeier W, et al. Long-term antithrombotic protection by in vivo depletion of platelet glycoprotein VI in mice. *J Exp Med.* Feb 19 2001;193(4):459-469.

153. Arthur JF, Dunkley S, Andrews RK. Platelet glycoprotein VI-related clinical defects. *Br J Haematol.* Nov 2007;139(3):363-372.

154. Loyau Inserm S, Faille D, Gautier P, Nurden P, Jandrot-Perrus M, Ajzenberg N. Absence of bleeding upon dual antiplatelet therapy in a patient with a immune GPVI deficiency. *Platelets.* Jul 5 2020:1-5.

155. Nurden P, Tandon N, Takizawa H, et al. An acquired inhibitor to the GPVI platelet collagen receptor in a patient with lupus nephritis. *J Thromb Haemost.* Sep 2009;7(9):1541-1549.

156. Dunkley S, Arthur JF, Evans S, Gardiner EE, Shen Y, Andrews RK. A familial platelet function disorder associated with abnormal signalling through the glycoprotein VI pathway. *Br J Haematol.* Jun 2007;137(6):569-577.

157. Sugiyama T, Okuma M, Ushikubi F, Sensaki S, Kanaji K, Uchino H. A novel platelet aggregating factor found in a patient with defective collagen-induced platelet aggregation and autoimmune thrombocytopenia. *Blood.* Jun 1987;69(6):1712-1720.

158. Jandrot-Perrus M, Hermans C, Mezzano D. Platelet glycoprotein VI genetic quantitative and qualitative defects. *Platelets.* 2019;30(6):708-713.

159. Matus V, Valenzuela G, Saez CG, et al. An adenine insertion in exon 6 of human GP6 generates a truncated protein associated with a bleeding disorder in four Chilean families. *J Thromb Haemost.* Sep 2013;11(9):1751-1759.

160. Nagy M, Perrella G, Dalby A, et al. Flow studies on human GPVI-deficient blood under coagulating and noncoagulating conditions. *Blood Adv.* Jul 14 2020;4(13):2953-2961.

161. Kato K, Kanaji T, Russell S, et al. The contribution of glycoprotein VI to stable platelet adhesion and thrombus formation illustrated by targeted gene deletion. *Blood.* Sep 1 2003;102(5):1701-1707.

162. Massberg S, Gawaz M, Gruner S, et al. A crucial role of glycoprotein VI for platelet recruitment to the injured arterial wall in vivo. *J Exp Med.* Jan 6 2003;197(1):41-49.

163. Lockyer S, Okuyama K, Begum S, et al. GPVI-deficient mice lack collagen responses and are protected against experimentally induced pulmonary thromboembolism. *Thromb Res.* 2006;118(3):371-380.

164. Cheli Y, Jensen D, Marchese P, et al. The Modifier of hemostasis (Mh) locus on chromosome 4 controls in vivo hemostasis of Gp6-/- mice. *Blood.* Feb 1 2008;111(3):1266-1273.

165. Sarratt KL, Chen H, Zutter MM, Santoro SA, Hammer DA, Kahn ML. GPVI and alpha2beta1 play independent critical roles during platelet adhesion and aggregate formation to collagen under flow. *Blood.* Aug 15 2005;106(4):1268-1277.

166. Jung SM, Tsuji K, Moroi M. Glycoprotein (GP) VI dimer as a major collagen-binding site of native platelets: direct evidence obtained with dimeric GPVI-specific Fabs. *J Thromb Haemost.* Aug 2009;7(8):1347-1355.

167. Loyau S, Dumont B, Ollivier V, et al. Platelet glycoprotein VI dimerization, an active process inducing receptor competence, is an indicator of platelet reactivity. *Arterioscler Thromb Vasc Biol.* Mar 2012;32(3):778-785.

168. Jamasbi J, Megens RT, Bianchini M, et al. Differential Inhibition of Human Atherosclerotic Plaque-Induced Platelet Activation by Dimeric GPVI-Fc and Anti-GPVI Antibodies: Functional and Imaging Studies. *J Am Coll Cardiol.* Jun 9 2015;65(22):2404-2415.

169. Burkhart JM, Vaudel M, Gambaryan S, et al. The first comprehensive and quantitative analysis of human platelet protein composition allows the comparative analysis of structural and functional pathways. *Blood.* Oct 11 2012;120(15):e73-82.

170. Best D, Senis YA, Jarvis GE, et al. GPVI levels in platelets: relationship to platelet function at high shear. *Blood.* Oct 15 2003;102(8):2811-2818.

171. Bigalke B, Stellos K, Geisler T, et al. Expression of platelet glycoprotein VI is associated with transient ischemic attack and stroke. *Eur J Neurol.* Jan 2010;17(1):111-117.

172. Al-Tamimi M, Gardiner EE, Thom JY, et al. Soluble glycoprotein VI is raised in the plasma of patients with acute ischemic stroke. *Stroke.* Feb 2011;42(2):498-500.

173. Barrachina MN, Sueiro AM, Izquierdo I, et al. GPVI surface expression and signalling pathway activation are increased in platelets from obese patients: Elucidating potential anti-atherothrombotic targets in obesity. *Atherosclerosis.* Feb 2019;281:62-70.

174. Ollikainen E, Mikkelsson J, Perola M, Penttila A, Karhunen PJ. Platelet membrane collagen receptor glycoprotein VI polymorphism is associated with coronary thrombosis and fatal myocardial infarction in middle-aged men. *Atherosclerosis.* Sep 2004;176(1):95-99.

175. Croft SA, Samani NJ, Teare MD, et al. Novel platelet membrane glycoprotein VI dimorphism is a risk factor for myocardial infarction. *Circulation.* Sep 25 2001;104(13):1459-1463.

176. Bigalke B, Lindemann S, Ehlers R, et al. Expression of platelet collagen receptor glycoprotein VI is associated with acute coronary syndrome. *Eur Heart J.* Sep 2006;27(18):2165-2169.

177. Gao S, Han Y, Chen X, et al. Epigenetic modulation of glycoprotein VI gene expression by DNA methylation. *Life Sci.* Jan 15 2020;241:117103.

178. Zahid M, Mangin P, Loyau S, et al. The future of glycoprotein VI as an antithrombotic target. *J Thromb Haemost.* Dec 2012;10(12):2418-2427.

179. Jiang P, Jandrot-Perrus M. New advances in treating thrombotic diseases: GPVI as a platelet drug target. *Drug Discov Today.* Sep 2014;19(9):1471-1475.

180. ClinicalTrials.gov. Intracoronary Stenting and Antithrombotic Regimen: Lesion Platelet Adhesion as Selective Target of Endovenous Revacept (ISAR-PLASTER). 2017; https://clinicaltrials.gov/ct2/show/NCT03312855. Accessed Dezember 08,2020.

181. Mayer K, Hein-Rothweiler R, Schupke S, et al. Efficacy and Safety of Revacept, a Novel Lesion-Directed Competitive Antagonist to Platelet Glycoprotein VI, in Patients Undergoing Elective Percutaneous Coronary Intervention for Stable Ischemic Heart Disease: The Randomized, Double-blind, Placebo-Controlled ISAR-PLASTER Phase 2 Trial. *JAMA Cardiol.* Mar 31 2021.

182. ClinicalTrials.gov. Revacept in symptomatic carotid stenosis. 2012; https://clinicaltrials.gov/ct2/show/NCT01645306. Accessed Dezember 08,2020.

183. Seizer P, Borst O, Langer HF, et al. EMMPRIN (CD147) is a novel receptor for platelet GPVI and mediates platelet rolling via GPVI-EMMPRIN interaction. *Thromb Haemost.* Apr 2009;101(4):682-686.

184. Mammadova-Bach E, Ollivier V, Loyau S, et al. Platelet glycoprotein VI binds to polymerized fibrin and promotes thrombin generation. *Blood.* Jul 30 2015;126(5):683-691.

185. Alshehri OM, Hughes CE, Montague S, et al. Fibrin activates GPVI in human and mouse platelets. *Blood.* Sep 24 2015;126(13):1601-1608.

186. Onselaer MB, Hardy AT, Wilson C, et al. Fibrin and D-dimer bind to monomeric GPVI. *Blood Adv.* Aug 22 2017;1(19):1495-1504.

187. Mangin PH, Onselaer MB, Receveur N, et al. Immobilized fibrinogen activates human platelets through glycoprotein VI. *Haematologica.* May 2018;103(5):898-907.

188. Induruwa I, Moroi M, Bonna A, et al. Platelet collagen receptor Glycoprotein VI-dimer recognizes fibrinogen and fibrin through their D-domains, contributing to platelet adhesion and activation during thrombus formation. *J Thromb Haemost.* Feb 2018;16(2):389-404.

189. Talens S, Leebeek FW, Demmers JA, Rijken DC. Identification of fibrin clot-bound plasma proteins. *PLoS One.* 2012;7(8):e41966.

190. Nikolajsen CL, Dyrlund TF, Poulsen ET, Enghild JJ, Scavenius C. Coagulation factor XIIIa substrates in human plasma: identification and incorporation into the clot. *J Biol Chem.* Mar 7 2014;289(10):6526-6534.

191. Lorand L. Factor XIII: structure, activation, and interactions with fibrinogen and fibrin. *Ann N Y Acad Sci.* 2001;936:291-311.

192. Zhang D, Ebrahim M, Adler K, et al. Glycoprotein VI is not a Functional Platelet Receptor for Fibrin Formed in Plasma or Blood. *Thromb Haemost.* Jun 2020;120(6):977-993.

193. Gawaz M. Novel Ligands for Platelet Glycoprotein VI. *Thromb Haemost.* Mar 2018;118(3):435-436.

194. Chen C, Rawat D, Samikannu B, Bender M, Preissner KT, Linn T. Platelet glycoprotein VI-dependent thrombus stabilization is essential for the intraportal engraftment of pancreatic islets. *Am J Transplant.* Oct 25 2020.

195. Ahmed MU, Kaneva V, Loyau S, et al. Pharmacological Blockade of Glycoprotein VI Promotes Thrombus Disaggregation in the Absence of Thrombin. *Arterioscler Thromb Vasc Biol.* Sep 2020;40(9):2127-2142.

196. Montague SJ, Delierneux C, Lecut C, et al. Soluble GPVI is elevated in injured patients: shedding is mediated by fibrin activation of GPVI. *Blood Adv.* Feb 13 2018;2(3):240-251.

197. Montague SJ, Hicks SM, Lee CS, et al. Fibrin exposure triggers alphaIIbbeta3-independent platelet aggregate formation, ADAM10 activity and glycoprotein VI shedding in a charge-dependent manner. *J Thromb Haemost.* Jun 2020;18(6):1447-1458.

198. Auger JM, Kuijpers MJ, Senis YA, Watson SP, Heemskerk JW. Adhesion of human and mouse platelets to collagen under shear: a unifying model. *Faseb J.* May 2005;19(7):825-827.

199. Herr AB, Farndale RW. Structural insights into the interactions between platelet receptors and fibrillar collagen. *J Biol Chem.* Jul 24 2009;284(30):19781-19785.

200. Kuijpers MJ, Schulte V, Bergmeier W, et al. Complementary roles of glycoprotein VI and alpha2beta1 integrin in collagen-induced thrombus formation in flowing whole blood ex vivo. *Faseb J.* Apr 2003;17(6):685-687.

201. Gruner S, Prostredna M, Aktas B, et al. Anti-glycoprotein VI treatment severely compromises hemostasis in mice with reduced alpha2beta1 levels or concomitant aspirin therapy. *Circulation.* Nov 2 2004;110(18):2946-2951.

202. Mosesson MW. Fibrinogen and fibrin structure and functions. *J Thromb Haemost.* Aug 2005;3(8):1894-1904.

203. Lord ST. Molecular mechanisms affecting fibrin structure and stability. *Arterioscler Thromb Vasc Biol.* Mar 2011;31(3):494-499.

204. Ebrahim M, Jamasbi J, Adler K, et al. Dimeric Glycoprotein VI Binds to Collagen but Not to Fibrin. *Thromb Haemost.* Feb 2018;118(2):351-361.

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