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Review

Blood platelet surface receptor genetic variation and risk of thrombotic episodes

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ABSTRACT

Haemostasis is a set of processes whose main task is to prevent blood loss by creating barriers in damaged vessels. Because of the large number of platelet surface receptors and their many agonists, platelets can be activated in normal and pathologic states leading to thromboembolic complications. Although age, blood pressure, LDL and HDL, diabetes, lack of physical activity, obesity and stress are well established risk factors, recent work has shown that platelet receptor polymorphisms also impact platelet function. The most common polymorphisms include 14A/T (PAR-1), 139C/T, 744T/C, 52G/T, i-ins801A (P2Y12), 1622A/G, −5T/C (GPIbα) 1565C/T (GPIIb/IIIa) and 807C/T (GPIa/IIa). This review examines the influence of these polymorphisms on cardiovascular disease including myocardial infarction, deep venous thromboembolism and acute coronary syndromes. Elucidation of these genetic variations will facilitate our understanding of the complex molecular mechanisms involved with physiologic and pathophysiologic platelet activation and clot formation.

1. Introduction

Blood platelets are the smallest, un-nucleated morphotic elements of the blood that play a key role in maintaining normal haemostasis, i.e. the physiological balance between the processes of pro- and anti-coagulation. Platelets are formed from fragments of the bone marrow's megakaryocytic cytoplasm. They have a discoidal shape and quantity ranging from 140 to 440*10^9/L. The vitality of the platelet counts lasts from 8 to 12 days. They are removed from the bloodstream by the reticuloendothelial system in the spleen in the phagocytosis process [1].

Platelets are surrounded by a cell membrane that forms two layers of lipids in which proteins are distributed. Phospholipids, such as phosphatidylserine, phosphatidylethanolamine and others, dominate among the lipids. Some proteins passing through both layers of the cell membrane are linked to oligosaccharides, thus forming glycoproteins, which are receptors for many of the factors that activate or inhibit platelet function [2].

The platelet cytoskeleton is responsible for maintaining the shape of the platelet and its changes during activation. The primary ingredients of the cytoskeleton are alpha and beta tubulin, actin polymers, and proteins mediating their association. In the construction of blood platelets, we can also distinguish intracellular structures such as: an open canalicular system (OCS); a dense tubular system for storage of calcium ions; enzymes directing the transformation of arachidonic acid; single mitochondria; glycogen particles; peroxisomes, and some Golgi's apparatus. Numerous specific granules dispersed in the cytoplasm include: α-granules, containing platelet factor 4; fibrinogen; von Willebrand factor (vWF) or B-thromboglobulin, and various dense granules in which ADP, ATP, serotonin, and catecholamines are stored [1,2].

Platelets play a pivotal role in primary and secondary haemostasis. In primary haemostasis, the first stage is known as adhesion and sees the indirect binding of GP Ib/IX/V and IIb/IIIa glycoprotein to the damaged vessel by vWF factor and other platelet glycoproteins. These bind with the collagen in the subendothelial matrix. Releasing the biologically active substances from a platelet's granules causes recruitment of further platelets, thereby enhancing their activation and aggregation process [2]. Specialised glycoprotein receptors provide platelets for adhesion to the proteins, which are exposed in areas of vascular damage. The process of adhesion and/or the interaction of soluble agonists with receptors on the platelet activates the platelet integrin receptors to fibrinogen, and then the platelets are able to aggregate together. This aggregation creates a platelet plug that seals the breach in the vessel wall and prevents from excess blood loss [3]. Activated platelets then facilitate secondary haemostasis, the formation of a fibrin clot, by carrying coagulation factors and providing a catalytic surface for the major interactions of the coagulation cascade [4,5]. Activation of the blood platelets is a multi-step process in which various responses occur: shape change; adherence to vessel walls; secretion of

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biologically active compounds from granules; aggregation; expression of P-selectin; phosphorylation of specific proteins; exposure of anionic phospholipids on the extracellular surface of the platelet membrane, and release of microparticles rich in pro-coagulant activity. Morphological changes are accompanied by biochemical pathways: the enzymatic cascade of arachidonic acid, the change in concentration of cAMP and cGMP, activation of kinases and phosphorylation of proteins, the formation of reactive oxygen species (ROS) and the transformation of phosphatidylinositols [6,7]. Despite the absence of the nucleus, platelet activation is very complicated, and is associated with signal transduction through a number of surface receptors in the cell membrane of platelets combined with elements of enzymatic signal transduction chains [1].

Because of the large number of specific membrane receptors, blood platelets are highly reactive cells, readily activated by many physiological and unphysiological agonists [1]. Signalling pathways via specific receptors are dependent on the type of agonist, but always lead to physiological responses expressed as platelet activation. Platelet activation mediated by the complex series of intracellular processes involved in haemostasis, thrombosis, and inflammation is the most critical risk factor in cardiovascular system disturbance, and is associated with the occurrence of thromboembolic complications [8]. Thromboembolic complications lead to acute ischaemic coronary syndromes, stroke, and deep vein thrombosis, are a cause of death or chronic conditions that limit quality of life and generate high therapy and care costs.

2. The role of platelets in acute coronary syndromes

Acute coronary syndrome (ACS) is any group of clinical symptoms compatible with acute myocardial ischemia. ACS includes unstable angina (UA), non-ST-segment elevation myocardial infarction (NSTEMI), and ST-segment elevation myocardial infarction (STEMI) [9]. It is well known that acute coronary syndromes with different clinical manifestations have a common pathophysiology, which is associated with coronary artery thrombosis [10]. Platelets are known to play a fundamental role in acute coronary syndromes. Platelets can form pathogenic, occlusive intracoronary thrombus, leading to acute ischaemic events. Platelet adhesion and aggregate formation are critical events that occur in acute coronary syndromes [11].

In the last few years, researchers have described the presence of activated platelets in systemic circulation in various cardiovascular disease states, particularly acute coronary syndromes [12]. Patients with acute coronary syndromes have increased activity and aggregation of blood platelets inside coronary circulation, resulting in partial or complete obstruction of the coronary artery [13]. Platelets contribute to acute thrombosis via a multi-step mechanism. The first step is adhesion to the endothelium. The constituents of the exposed subendothelium then interact, including collagen, vWF, fibronectin, and specific platelet surface membrane receptors. Thus, the platelets overcome the high blood-shear forces and attach themselves to the target endothelium site. After attachment, the platelets present an activation process with a specific conformational change that induces the onset of multiple internal signalling networks. Hyper-reactive platelets accelerate the formation of an intracoronary thrombus, leading to a cascade of clinical events [14].

A recent study evaluated the association between the hyper-reactivity of platelets to ADP and outcomes in patients with stable cardiovascular disease [15]. However, platelets don't only play a role in the formation of coronary artery thrombosis – many types of research indicate that platelets could also play an essential role in the initiation and propagation of atherosclerosis, potentially through interaction of activated platelets with endothelial cells and leukocytes, or by stimulating inflammation through the release of various mediators [16]. Atherosclerotic lesions rely on the creation of atherosclerotic plaque in medium or large arteries, which form a surface for the formation of thrombi. This process leads to a reduction in the lumen of the blood vessels, resulting in partial or complete obstruction. The generation of atherosclerotic plaque can be an effect of hypercholesterolaemia, diabetes, smoking and hypertension. These factors can cause dysfunction of the endothelium, the disrupted functioning of which plays a major role in the process of atherosclerosis. All complicated interactions between the endothelium, inflammatory cells and platelets, contribute to the pathogenesis of ACS. Ruptures of atherosclerotic plaque could be the cause of two-thirds of all cases of ACS. This process is dependent on many factors, such as lipid content of the plaque, blood flow, the prothrombotic and antithrombotic balance in patients, the stability of the atherosclerotic plaque and intensity of the inflammation [9]. Activated platelets release an arsenal of potent inflammatory and mitogenic substances into the local microenvironment, thereby altering the chemotactic, adhesive and proteolytic properties of endothelial cells. These platelet-induced alterations of the endothelial phenotype support chemotaxis, adhesion and the transmigration of monocytes to the site of inflammation. Different mediators, such as adhesion proteins (e.g. fibrinogen, fibronectin, vWF, thrombospondin, vitronectin, P-selectin, GPIIb-IIIa), growth factors (e.g. PDGF, TGF-β, EGF, bFGF), chemokines (e.g. RANTES, platelet factor 4 [PF4], CXC chemokine ligand 4 [CXCL4]), epithelial neutrophil-activating protein 78 (ENA-78; CXCL5), cytokine-like factors (e.g. IL-1β, CD40L, β-thromboglobulin) and coagulation factors (e.g. factors V, XI, PAI-1, plasminogen, protein S) are released from dense granules, α-granules, lysosomes, canalicular system, and the cytosol of activated platelets. These proteins act in a concerted and fine-regulated manner, influencing a wide variety of biological functions, including cell adhesion, cell aggregation, chemotaxis, cell survival and proliferation, coagulation, and proteolysis – all of which accelerate inflammatory processes and cell recruitment [17].

3. G Protein-coupled receptors in blood platelets

G Protein-Coupled Receptors (GPCR) constitute the largest family of proteins in the human genome responsible for signal transmission through the lipid bilayer to the effector sites in cells. Comparative analysis of GPCR receptor sequences has led to their division into five main families: Rhodopsin, Glutamate, Adhesion, Frizzled/Taste2 and Secretin [18].

GPCR receptor sequences can be activated by a very chemically diverse group of ligands, including amines, lipids, peptides, ions, nucleotides and proteases. Because of their ability to specifically interact with various functionally different heterotrimeric guanine nucleotide-binding proteins (G proteins), agonist-activated GPCRs can induce different signalling pathways to change their cellular function.

The Gq/G11 family of G proteins couple receptors to the β isoforms of phospholipase C (PLC), of which the β2 and β3 isoforms are particularly present in platelets. Activation of PLC results in the formation of IP3 and diacylglycerol, leading to elevation of free cytoplasmic Ca^{2+} and activation of protein kinase C (PKC), respectively. Although most cells in the mammalian organism express both Gq and G11, platelets are an exception to that, containing only Gq. So far, no physiological significance for the lack of G11 in platelets has been reported. G13, a member of the G12/G13 family, has been shown to regulate several signalling pathways of which the Rho/Rho-kinase-mediated pathway is the most well established. Activated G13 binds and activates a subgroup of Rho-specific guanine nucleotide exchange factors. Gi2, the main member of the Gi family expressed on platelet-coupled receptors in an inhibitory fashion to adenylyl cyclase. In addition, Gi-type G proteins are a significant source of βγ complexes, which are released upon G-protein activation and can regulate a variety of channels or enzymes, including adenylyl cyclase and phosphatidylinositol 3-kinases (PI3K). The latter enzyme produces phosphatidylinositol-3,4,5-trisphosphate, which activates a variety of downstream effectors including the serine/threonine kinase Akt/protein kinase B (PKB) [19].

The GPCR family comprises the most extensive class of

pharmaceutically relevant target molecules, with > 30% of the total drugs on the market targeting GPCRs. The great versatility of the G protein-mediated signalling system could explain why it is the primary mediator of the second phase of platelet activation during thrombosis and haemostasis, which requires the fast, coordinated action of a variety of diffusible mediators to activate platelets and recruit them into the growing thrombus [19].

Amisten et al. [20] detected in human platelets mRNA of 28 GPCR genes. Of the 28 verified GPCRs mRNA expressed in platelets, 12 genes could be quantified. The expression level of evaluated genes were normalized to P2Y1, whose expression is defined as 1, and fold-change was converted into percentages. The thrombin receptor PAR1 (1865 ± 178%) was the most abundant GPCR mRNA expressed on platelets, followed by the ADP receptor P2Y12 (459 ± 88%), succinate receptor 1 (257 ± 48%) and the ADP receptor P2Y1 (100%; P2Y1 was chosen as calibrator).

Thrombin is one of the strongest in-vivo platelet agonists. Platelet activation occurs at a much lower concentration of thrombin than is needed for the fibrinogen to fibrin conversion. Thrombin-activated platelets change their shape, secrete the contents of their granules and finally aggregate [21]. Receptors of the Protease-Activated Receptor (PAR) family are responsible for platelets' response to thrombin. Thrombin's attachment with PAR's N-terminus causes its cleavage, thus this terminus binds permanently with the second loop of PAR. PAR1 and PAR4 are present on the human platelets' surface receptors. PAR1 activation occurs at lower concentrations of thrombin than is required in the case of receptor PAR4. Therefore, platelet activation is responsible mainly for PAR1, whereas PAR4 only has ancillary functions. The number of copies of the PAR1 receptor on the blood platelets' surface ranges from 1500 to 2000. Thrombin cleaves the N-terminal extracellular domain of PAR to expose a new N-terminus, which binds to the central extracellular loop of the same receptor, causing its activation and initiating the intracellular signalling events. The PAR1 receptor is associated with three G proteins; G13, Gq and Gi. Thus, activation of the PAR1 receptor can result in a platelet response, dependent on a variety of intracellular signal transduction pathways [22,23].

For the PAR1 receptor, three polymorphisms are described: 1426C/T, 506I/D and 14A/T. In contrast to 14A/T polymorphism, which is related with PAR1 expression level, studies show that 1426C/T and 506I/D polymorphism are not associated with PAR1 phenotype. Analysis of the platelets' PAR1 genotype according to the PAR1 phenotype shows that the polymorphism 14A/T is associated with platelet response to the thrombin receptor activating peptide – SFLLRN (TRAP). Homozygous carriers of the 14A allele have a significantly augmented level of PAR1 than heterozygous carriers. Volunteers with two copies of 14A allele also had a higher expression level of P-selectin than heterozygous. It has been suggested that the phenotype effect of 14A/T polymorphism of the PAR1 receptor could be related with intron splicing, because of its location in intervening sequence (IVS), 14 nucleotides above the exon 2 start site (Table 1) [24].

Zhang et al. [25] showed that polymorphism 14A/T could significantly contribute to the risk of ischaemic events in Chinese patients with ST-elevation myocardial infarction. Patients with at least one T allele had a meaningfully lower risk of ischaemic disease than homozygous carriers of the 14A allele. The Zhang et al. study showed that reduced expression of PAR1 results in decreased platelet reactivity, thus decreasing the risk of ischaemic disease in patients with T allele [25,26].

Table 1
Phenotype effects of 14A/T polymorphism of PAR-1 receptor in platelets in 100 male volunteers.

Genotype	PAR-1 levels in platelets	Platelet secretory response to SFLLRN	P-selectin expression on the platelet surface
AA	1297 ± 186	11,548 ± 1735	11,548 ± 1735
AT/TT	1164 ± 203	10,160 ± 2117	10,530 ± 2078
p value	*0.013*	*0.001*	*0.011*

The ability of ADP to promote platelet aggregation has been recognized for nearly half a century. Platelets release dense granules that contain the nucleotide adenosine diphosphate which activates other platelets, ADP is also passively released from damaged erythrocytes and endothelial cells. Platelet activation by ADP is mediated by two G protein-coupled receptors, P2Y1 and P2Y12. Whereas P2Y1 couples to Gq, P2Y12 couples to the Gi type of G proteins. Studies using receptor agonists have suggested that activation of both receptors is required for a full response by platelets to ADP. P2Y1 is the pathway that initiates platelet activation, while P2Y12 plays a role in strongly amplifying the activation process. The P2Y1 receptor initiates platelet shape change and ADP-induced aggregation through the mobilisation of internal calcium stores, and the P2Y12 receptor, which is coupled to adenylyl cyclase inhibition, is essential for a full aggregation response to ADP and the stabilisation of aggregates [19]. This amplifies not only platelet aggregation but also expands the other functional consequences of activation, including granule release and platelet pro-coagulant activity. Deletion of either P2Y1 or P2Y12 in mice prolongs their bleeding time and impairs platelet responses, not only to ADP, but also to thrombin and TXA2, particularly in low concentrations [26]. Platelet responses to thrombin and TXA2 in low and intermediate concentrations are reduced in the absence of ADP receptors. Since platelet TXA2 receptors do not couple directly to Gi family members, platelet aggregation induced by TXA2 requires the secretion of ADP to inhibit adenylyl cyclase. Both P2Y1 and P2Y12 are involved in ADP-induced platelet pro-coagulant activity. Over the past decade, ADP receptors on the platelet membrane have also become a target for antithrombotic strategies with thienopyridines. Two existing thienopyridine compounds, Ticlopidine and Clopidogrel, have irreversibly inhibited P2Y12 and have been shown to have clinically useful antiplatelet activity for the secondary prevention of cardiovascular events [26].

In healthy donors, four genetic polymorphisms of P2Y12 receptor have been described, including three SNPs (139C/T, 744 T/C, 52G/T), and one nucleotide insertion in the receptor's gene (i-ins801A). This establishes two haplotypes: H1, with C in position 139, T in position 744, G in position 52 and a lack of i-ins801A; and H2, with T in position 139, C in position 744, T in position 52 and the presence of the i-ins801A. The frequencies of those haplotypes were 86% for H1 and 14% for H2 [26,27]. Fontana et al. [27] presented a study in the healthy subjects of which ADP-induced platelet aggregation was associated with an H2 haplotype of the P2Y12 receptor gene (OR = 3.3 [1.1–10.4]). In contrast, in a study conducted by Shu-Jun et al. on a Han Chinese population, the association between haplotypes and the presence of cerebral infarction was not significant ($p > .05$), but the H2 allele frequency was meaningfully higher in patients with cerebral infarction (14.5%), than in the healthy controls (8.6%). The authors suggested that the results obtained may be linked with H2 haplotype distribution, which varies depending on the race of the subjects: 8.6% in the Han Chinese population, 13.8% a in Caucasian population, and 12.8% in a Mexican population (Fig. 1) [28].

Zoheir et al. had a similar result, in which carriers of the C allele in 744 T/C were associated with augmented platelet activation in response to ADP. This suggests that there is a positive correlation between P2Y12 receptor and platelet reactivity [29]. Further, although the 744 T/C polymorphism of the P2Y12 receptor gene has been associated with enhanced platelet aggregation in healthy volunteers, it has not shown any influence on clopidogrel response assessed by ADP [30]. The 744 T/C polymorphism of the P2Y12 receptor gene also does not modulate platelet response to Clopidogrel, either in the early or long-term phases of treatment. Several P2Y12 polymorphisms were found by Hetherington et al. [31], but none of them influenced the reactivity of platelets. In addition, according to Fontana's study, Hetherington et al. did not see differences between haplotypes. The reason for the

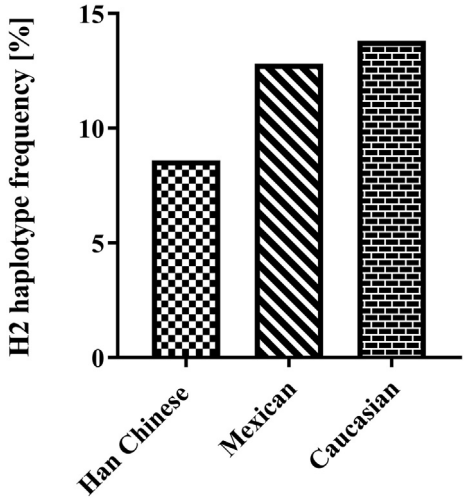

Fig. 1. Haplotype H2 frequency in different Han Chinese, Mexican and Caucasian population [28].

divergences in the results are unclear, although the difference in the age of the volunteers is suspected (Fontana's et al. volunteers had lower age) [31]. In addition, Zhang et al. [25] showed that 52G/T polymorphism is associated with bleeding events (OR = 2.71 [1.298–5.659]). The possible link between the H2 haplotype and augmented platelet aggregation in response to ADP remains unclear. Fontana et al. [27] suggested that the H2 haplotype increases the number of P2Y12 receptors on the platelet surface, and as such higher responsiveness to ADP can occur. They overrule the role of amino acid substitution and splice variants, because of the valid exon 1 to exon 2 junctions revealed by cDNA analysis.

P2Y1 gene variation 1622A/G has also been associated with platelet responsiveness to various ADP concentrations (0.1–10 μM). The response was 130% higher in GG homozygotes than in AA homozygotes when platelets were stimulated with 0.1 μM ADP. Increased platelet response for ADP affects the functioning of the GPIIb-IIIa, which is important in fibrinogen binding. 1622A/G is a silent variation which means, that this SNP is not affecting on the amino acid sequence in the genome and don't have an influence on receptor structure. The molecular mechanism explanation of 1622A/G SNP and platelet response to ADP remains unclear. Hetherington et al. suggested that this polymorphism may affect P2Y1 expression and play a potential role in regulating the promoter region [31]. However, in Fontana et al. [32], performed on 98 healthy Caucasian men, the 1622A/G polymorphism of the P2Y1 gene was only weakly associated with maximal ADP-induced aggregation in univariate analysis, and only if GG homozygotes were pooled with AG heterozygotes. In the same study, none of the P2Y12 variations were associated with platelet responsiveness to ADP. Additionally, Galic et al. [33] presented a study suggesting that 18C/T SNP of the P2Y12 gene may be an independent predictor of pharmacological response to Clopidogrel.

4. Platelet membrane glycoproteins

The initial interaction of platelets with the extracellular matrix involves the platelet vWF receptor GPIb/IX/V. During the next stage of platelet activation, a platelet plug forms through the recruitment of additional platelets from the circulation, and their integrin GPIIb/IIIa-mediated aggregation. GP Ib/IX/V complex, which is critical to platelet adhesion, especially in the initial events that tether the platelets to the subendothelial matrix, binds to vWF. This adhesive bond has a rapid dissociation rate, resulting in platelet translocation on the vessel wall [34]. vWF is multimeric glycoprotein found in 1924 by Erik von Willebrand. In humans, vWF contain 2813 amino acids with 741 amino acids pro-polypeptide and 22 amino acids signal peptide. The transformation of vWF into active form requires a cleavage of pro-polypeptide, resulting in formation of mature vWF containing 2050 amino acids. Analysis of the amino acid sequence shows that vWF has many active domains responsible for its cleavage and functions. For platelets interaction with the extracellular matrix, A1 domain is essential. It contains a binding site for GPIbα, a subunit of GP Ib/IX/V complex. The A2 domain contains the cleavage site and A3 domain contain a binding site for collagen. Very important role in haemostasis is also played by C1 domain, which is a binding site for GP IIb/IIIa (Fig. 2) [35].

Despite the fact that vWF is synthesized and stored in endothelium cells, platelets and megakariocytes, studies showed that most of the circulating vWF has endothelial origin, and platelet/megakaryocyte derived vWF constitute approximately 15%. Under physiological conditions, vWF do not interact with GPIbα. However, high shear forces in bloodstream results in immobilization of vWF and exposition of A1 domain, allowing to receptor-ligand interaction. What is more, binding the vWF with GPIbα results in exposition of GP IIb/IIIa which binds fibrinogen, thus increasing aggregation process [36]. Evidence indicating the significance of the functions performed by vWF is von Willebrand Disease (vWD), which is responsible for bleeding disorders caused by absence of vWF [37]. Above evidences shows that GP Ib/IX/V is essential in platelet adhesion to activate endothelial cells. Furthermore, GP Ib/IX/V is important in leukocyte recruitment to sites of vascular injury, and in thrombin-mediated platelet activation and coagulation, including microparticle formation. Recently, significant discoveries about the signalling pathways that regulate GP Ib/IX/V have been made; these pathways could be potential targets for antithrombotic drug development [38]. Kieffer et al. [39] described the biosynthesis of GPIb in blood platelets.

The GP Ib/IX/V complex plays an essential role in the initial phase of platelet-vessel wall interaction, which leads to the activation of integrin GP IIb/IIIa and contributes to GP IIb/IIIa-dependent platelet adhesion, spreading and aggregation [38].

Recently, a T/C polymorphism was identified in the Kozak sequence of glycoprotein Ibα gene at position −5 from the initiator ATG codons [40]. Kozak consensus sequence was originally described by Marilyn Kozak. His study revealed that all functional AUG triplets, known as start codons, are preceded by purine in position −3 and after AUG. Current state of knowledge shows that Kozak sequence is necessary for efficient initiation of translation process, because the recognition of this sequence by ribosomes constitute protein translational start site. Amino acid chain is identified by the following notation: (GCC)GCCAGCC**A UGG** [41]. The presence of the -5C allele increases the surface expression of GP Ib/IX/V complex, and it has been suggested that higher receptor levels might increase the adhesiveness of the platelets and confer the risk of thrombosis. Esen et al. [40] suggest that GPIbα T/C polymorphism might increase the risk of ischaemic stroke, especially in those with an undetermined aetiology. Blood samples were taken from 231 patients following their first ischaemic stroke event, and from 220 healthy volunteers. After analysis, 156 patients were TT homozygous, 70 patients were heterozygous, and only five patients carried the CC genotype. In the ischaemic stroke group, at least one C allele was highly presented (32.5%), compared to the control group (23%) (OR = 0.61 [0.4–0.93]). Esen et al. compared TT genotypes with heterozygous carriers and the results were important (OR = 0.58 [0.37–0.89]). Data from the Vienna Stroke Registry shows that carriers of the CC genotype have a 3.5-fold higher risk of stroke, compared with TT homozygous and heterozygous. Esen et al. could not confirm this information because of the low number of CC genotype carriers. The homozygous TT Kozak genotype could be a significant factor in recognition of coronary artery disease completed by myocardial infarction. On the other hand, Croft et al. [42] postulate that Kozak sequence polymorphism of the GPIba gene is not a major risk factor for myocardial infarction (OR = 1.03 [0.78–1.36]).

Platelet integrin GPIIb/IIIa (αIIbβ3), also known as the platelet

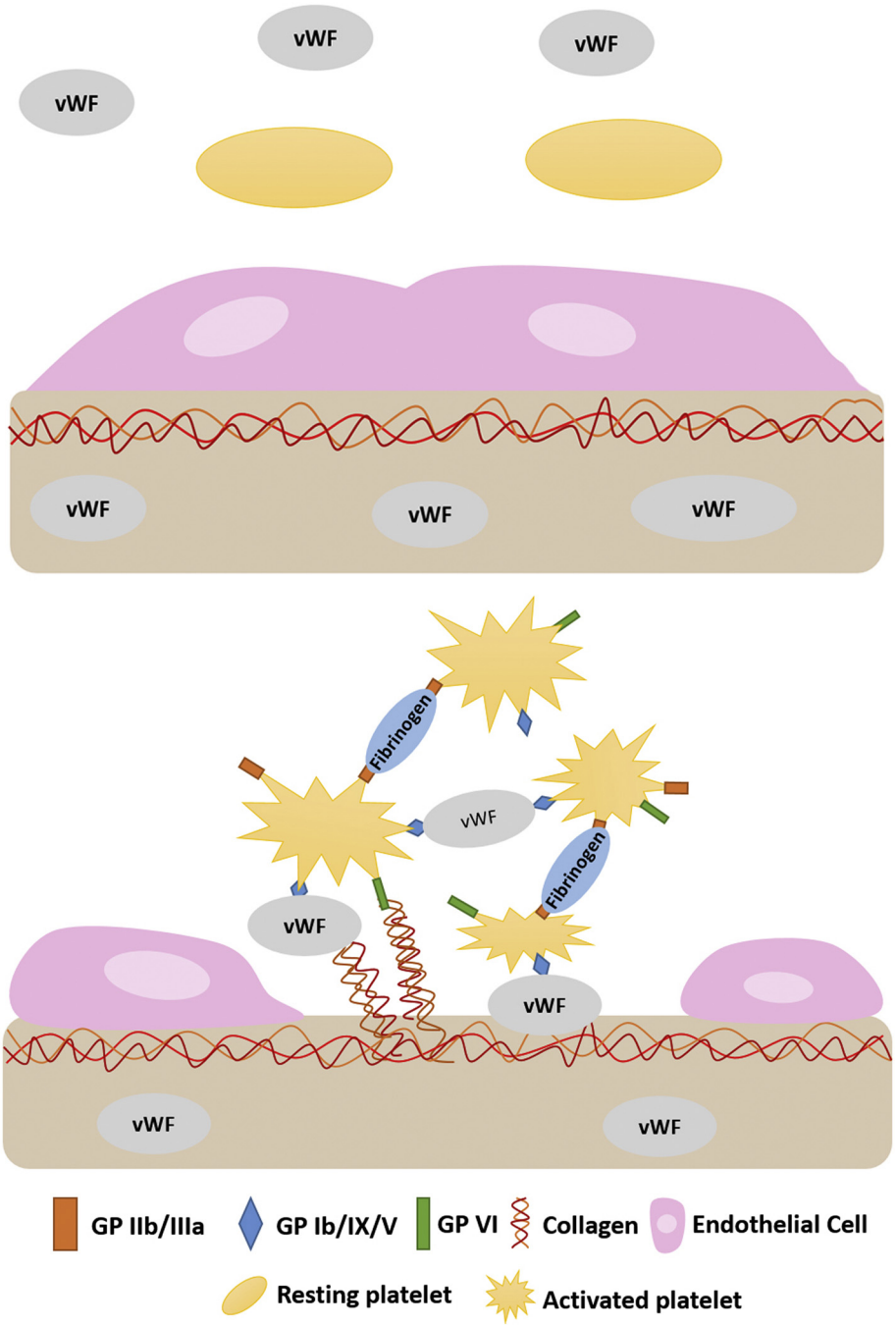

Fig. 2. The role of vWF in haemostasis. Vessel damage caused exposition of collagen and vWF contained in subendothelial matrix. Unique vWF domains bind with collagen (by A3 domain), GPIb/IX/V (by A1 domain) and GP IIb/IIIa (by C1 domain), resulting in blood platelets adhesion, shape change during activation, exposition of platelet surface receptors and aggregation.

fibrinogen receptor, is the major integrin on platelets and plays a crucial role in platelet accumulation on the activated endothelium – platelets spread and form a surface for the recruitment of additional platelets via fibrinogen bridges between two αIIbβ3 receptors. The complex is formed via the calcium-dependent association of subunits GPIIb and GPIIIa. GPIIb/IIIa is able to take two conformations; active and inactive. Only the active form is capable of binding protein ligands. Integrin activation is carried out by exposing the ligand molecule binding sites. A common feature of the ligands that bind to many integrins is the presence of the Arg-Gly-Asp (RGD) amino acid sequence, which was originally identified as the cell-binding domain of fibronectin. This RGD sequence exists in each of the four adhesive proteins that bind to GPIIb/IIIa: fibronectin, vWf, vitronectin, and fibrinogen. Integrin αIIbβ3 also reacts with the KQAGDV (Lys-Gln-Ala-Gly-Asp-Val) sequence, that is found only in the C-terminus of the fibrinogen γ chain [43]. GPIIb/IIIa is the most abundant integrin receptor on the platelet membrane, ranging from 50,000–80,000 copies per platelet. This glycoprotein is essential to platelet protein interactions in plasma and the extracellular matrix during both haemostasis and thrombosis. The GPIIb/IIIa receptor is a target for several antiplatelet drugs, including Abciximab, Eptifibatide, and Tirofiban. These antagonists block the IIb/IIIa receptors and prevent fibrinogen binding to this receptor, thereby blocking the final common pathway of platelet aggregation. They also block the 'outside-in' signalling that occurs during platelet aggregation and causes additional amplification of platelet activation. Defects in glycoprotein IIb/IIIa cause Glanzmann's

thrombasthenia. This is an extremely rare coagulopathy caused by a point mutation at position 119, in which the Asp residue is replaced by Tyr residue in GPIIIa glycoprotein [44]. As a result, fibrinogen bridging of platelets to other platelets cannot occur, and the bleeding time is significantly prolonged. In platelets, the presence of mRNA for both integrin subunits αIIb and β3 has been observed. This indicates that this receptor might be synthesized in the platelets [45]. During storage of platelets, the expression on surface GPIIb/IIIa increases after seven days by 13.4%, and after ten days by 41.9%. The total concentration of GPIIIa after seven days of storage is doubled, and after ten days is increased four-fold [46].

Glycoprotein IIb/IIIa receptors are polymorphic, with the two most common variants being PlA1 and PlA2 (by haematologists often called HPA-1a and HPA-1b respectively). These are caused by a single-point mutation of cytosine to thymidine in exon 2 of the GPIIIa gene, resulting in the substitution of proline (PlA1/HPA-1a) for leucine (PlA2/HPA-1b) at position 33, which results in a single nucleotide 1565C/T transition in the GPIIIa gene and may be associated with thrombotic cardiovascular complications [47]. PlA2 alleles might increase the risk of acute coronary syndromes. The association of the Leu33Pro polymorphism with the incidence of myocardial infarction was first reported by Marian et al. [48]. The strong association between the PlA2 polymorphism of the glycoprotein IIIa gene and acute coronary thrombosis was observed by Weiss et al. [49]. The relation between PIA2 and coronary events was higher in patients younger than 60 when their first coronary event appeared. Carriers of the PIA2 allele are more exposed to coronary heart disease than PIA1 homozygous. Weiss et al. examined 139 people and found that this association was most influential in patients who had unstable angina, or myocardial infarction, before the age of 60 (68 patients), in whom OR = 6.2 (1.8–22.4) compared to all ages OR = 2.8 (1.2–6.4). Adrissino et al. [50] showed that HPA-1b polymorphism is an influence on the higher risk of developing myocardial infarction at a young age. The odds ratio (1.89 [1.13–3.18]) in this study shows that among 200 patients, 54 had the HPA-1a/HPA-1b genotype compared to the control group, in which 33 volunteers had the same genotype. HPA-1b homozygosity is associated with a three- and four-times higher risk of ischaemic cardiovascular disease and myocardial infarction in young men [51]. Additionally, in a Scandinavian population polymorphism HPA-1b was significantly associated with an increased risk of myocardial infarction. Grove et al. [52] showed that the PIA2 allele was more frequent in patients who underwent myocardial infraction (187/529) than in patients who did not undergo MI (138/490) (OR = 1.4 [1.1–1.8]). PlA2 homozygosity was also associated with an inadequate response to aspirin therapy [53]. Kucharska-Newton et al. [54] also reported that polymorphism of the GPIIIa glycoprotein could be predisposed to an increased risk of atherosclerotic plaque rupture, and could be associated with changes in the structure of atherosclerotic plaques. Khatami et al. created a protein model of a normal and polymorphic human β3 chain. The model showed changes in protein structure, and N-terminal structure in a polymorphic protein that was disrupted [55]. However, there have been no demonstrations that can explain how 1565C/T SNP affects the phenotype of platelets. The only reasonable explanation for the increased risk of cardiovascular diseases was an augmented expression of P-selectin on the platelet surface in a patient with Leu33Pro substitution [54]. Due to the many sites at which the β3 chain was present, there is also evidence that 1565C/T polymorphism is associated with Autism Spectrum Disorders (ASD). Schuch et al. showed that proline substitution in the 33rd amino acid sequence could be related to aggression in ASD-diagnosed patients [OR = 2.931 (1.138–7.546)], and that if combined with other SNPs has an impact on echolalia and epilepsy incidents [56]. A study performed by Verdoia et al. [57] shows that PlA2 polymorphism of GPIIIa does not influence the prevalence and extent of angiographically-defined coronary artery disease in the general population, although it does play a role in younger patients. Due to the wide range of reports regarding the effect of Leu33Pro polymorphism and its impact on cardiovascular diseases, it is difficult to determine its unequivocal role in the pathogenesis of CVD.

The glycoprotein Ia/IIa complex, often called integrin α2β1, plays a pivotal role in collagen binding, which is an essential molecule involved in adhesion. Glycoprotein Ia/IIa consists of two subunits, α and β, whose mutual conformation creates the N-terminal extracellular globular head. This is a surface that binds ligands. The α subunits are composed of a seven-bladed β-propeller at the N-terminus [58]. The α2 subunit contains an exceptional domain (I domain), known as MIDAS (metal ion-dependent adhesion site), that binds Mg^{2+}/Mn^{2+} cations. Structural studies show that upon ligand binding, MIDAS and adjacent sides lead to a conformational change of the unique I-domain, from a closed to open condition. Experiments with soluble collagen and specific antibodies have shown that α2β1 can exist in multiple activation conditions. The evolutionarily-conserved unique β-cytoplasmic tail has two characteristic motifs, which recognize sequences for phosphotyrosine binding proteins (PTB). One of these, Talin, is known as a crucial integrin activator. As a result of Talin binding, the salt bridge between the two cytoplasmic integrin tails is disrupted. This consequently leads to a conformational change that shows the α2-I domain and β-A domain. Recent works show that Talin needs Kindlin, the second motif, to work properly. Kindlin binds to the β1-cytoplasmic tail, and despite normal expression of Talin, results in a lack of platelet adhesion to collagen [59]. The quantity of this integrin on platelet surfaces is highly variable, but its density is well known to be associated with 807C/T silent polymorphism (Table 2). In a study conducted by Kunicki et al. [60] the glycoprotein Ia/IIa level was differed significantly depending on genotype. Carriers of TT alleles have a higher density of GP Ia/IIa on platelet surfaces than CC homozygous carriers. This study also showed that the density level of GP Ia/IIa is inherited (the children of the patients in Kunicki's study also had augmented GPIa/IIa density on their platelets' surfaces). Due to the lack of changes in the amino acid sequence of the encoded glycoprotein, the explanation for this phenomenon remains unclear. Kunicki et al. suggested the potential role of other polymorphisms within the α2 gene that could have an impact on the promoter region. The relation between silent polymorphisms and other genetic mutations within the same gene region has been established for different inherited disorders, such as Gaucher disease. On the other hand, silent polymorphisms can affect mRNA stability, resulting in disproportions in their level. There are many works that shows the impact of 807C/T SNP on thrombotic episodes. Carlsson et al. reported results of their study conducted on 227 stroke diagnosed patients, proving that presence of T allele in 807C/T polymorphism may constitute an inherited risk factor of stroke in patients younger than 50 years old [OR = 3.02 (1.20–6.61)] [61]. Santoso et al. [62] postulate that 807C/T polymorphism is associated with the development of myocardial infarction and another coronary artery disease in a younger patient (in patients younger than 49 years old, OR = 2.61 (1.26–5.41) $p = .009$). In a study conducted by Reiner et al. [63] 807C/T SNP was related to augmented risk of ischaemic stroke in women. An increased density of GPIa/IIa might also have an impact on risk factors for cardiovascular diseases and platelet-dependent thromboembolic complications.

Dupont et al. [24] also describe differences in the timing of aggregation, at 10 s higher in the carriers of two C alleles (68 ± 16 s),

Table 2
The quantity of GPIa/IIa receptors on platelet surfaces, by genotype [24].

Genotype	Density
C/C	2810 +/− 756
C/T	3747 +/− 541
T/T	4434 +/− 449
p value	*< 0.001*

than in the carriers of two T alleles (58 ± 16 s). Bargahi et al. [58] showed that despite the influence of GPIa/IIa density caused by 807C/T, this SNP is not related to a higher risk of deep venous thromboembolism.

5. The link between genome-wide association study and cardiovascular disease

A genome-wide association study (GWAS) is an approach to the analysis of genotype frequencies in different diseases in a large population. It depends on fast scanning of the human genome to find an association between genetic variation and a particular disease. Discovery of new mutations is useful in explaining the molecular mechanisms of diseases that have not yet been thoroughly investigated [64].

The first reported association between SNPs and cardiovascular disease in a GWAS came from McPherson et al. [65]. In that study, the interval on chromosome 9p21 was scanned to indicate variations in the genome that contribute to the development of cardiovascular disease. They found two SNPs (rs10757274 and rs2383206) associated with cardiovascular disease. McPherson et al. suggested the potential role of these SNPs in promoting atherosclerotic plaque, thrombogenesis and augmented tendency of atherosclerotic plaque to rupturing [65]. This finding motivated other scientists to discover new genetic disorders having an impact on cardiovascular disease. In 2013, CARDIoGRAMplusC4D Consortium et al. [66] reported a study in which 63,746 patients with coronary artery disease and 130,681 controls participated. The authors of this study identified 15 statistically significant, new risk alleles associated with cardiovascular diseases. In 2016, Stitziel et al. [67] published the first exome-wide association study. The scientists identified novel mutations which had not previously been associated with coronary artery disease, in a study in which > 72,000 patients and 120,000 controls participated. Stitzel et al. found an association between protection from coronary artery disease and triglyceride levels which was lower in SNP carriers.

The number of SNPs and other genetic variations linked to cardiovascular disease is still growing. Kessler et al. [64] showed almost 60 different SNPs that have an impact on circulatory system disorders. GWAS is a rapidly growing field that is receiving more and more interest from scientists. All of the information obtained from GWAS has allowed researchers to find many future, promising, therapeutic targets. Discovering new variations in the human genome associated with cardiovascular disease can help with our understanding of the molecular process of pathological haemostasis.

6. Conclusions

Cardiovascular disease is one of the most common causes of death in the world. Increasing morbidity among young people without typical risk factors is a disturbing phenomenon, despite growing awareness of the need to live a healthy lifestyle, with proper physical activity and diet. This study shows that some people have an increased predisposition to cardiovascular disease, associated with changes in the human genome (Table 3) (Fig. 3). Many studies have shown that polymorphisms of platelet receptors affect the receptor's expression, density, reactivity and functioning, leading to increasing platelet aggregation and activation resulting in the formation of blood clots in vessels. On the other hand, according to the GWAS central database (https://www.gwascentral.org/), the SNPs described by us have been examined in cardiovascular disease, but unfortunately did not show statistically significant results (Table 4).

Explaining why the polymorphisms described in this work have not been included in large-scale GWAS research remains unclear. We are not sure whether the problem lies with small or poorly matched populations. In a study conducted by Matarin et al. patients were collected from The Ischaemic Stroke Genetics Study and were not excluded for typical cardiovascular disease risk factors (obesity, diabetes, smoking etc), but on the basis of diagnosed diseases (such as Alzheimer's, autism and Parkinson's), thus reducing the impact of genetic factors on the occurrence of ischaemic events [68]. Furthermore, cases were collected from 5 medical centres all over the United States on both Caucasian and African Americans, indicating large diversity in the population. Allele frequency dependent on ethnicity may be a reason for the variable results. We also did not find any data on patients who did not survive their ischaemic events. Perhaps the SNPs listed by us had a large impact on this? Besides the fact that unknown point mutations found in the coding regions of the described genes might refer to stroke or other significant changes in gene expression elements, GWAS studies focuses on more high-performance candidate genes. Characterized by low effect size, GWAS studies identify common variants, rarely showing specific variants in a gene, which results in some genetic variants remaining hidden. GWAS studies are able to nominate candidate genes for complex diseases, but usually don't identify causative alleles. These causative variants could contribute to general disease receptivity at a single locus. Massive populations and genetic variants evaluated in GWAS studies are burdened with a higher risk of false positives or negative results. Another interpretation of the variable results is linked with variation in the link between disequilibrium structures in the studied populations. The presence of causative alleles close to a particular locus could signal the association of a selected locus with the occurrence of cardiovascular disease. This is why reproducing the obtained results is important to confirming the results [69,70]. On the other hand, for various reasons epidemiological studies could contain unique populations, for instance, a particular socioeconomic stratum, or the presence of only one sex. Using such a rigorous selection of patient types could affect the ability to generalize results from experiments linked with genetic association. To conclude, the experimental works described by us indicate the ambiguous association of the described SNPs with the frequency of occurrence of various disease entities, and their possible phenotypic effect. The influence of platelet surface receptors' polymorphisms on the presence of cardiovascular disease remains unclear, but they could be the basis for their selection in more precise, future studies. If the polymorphisms presented in this study are inherited risk factors for myocardial infarction and other complications in the circulatory system, in the future we could predict the chance of these diseases occurring in young people.

Table 3
Main platelet receptors polymorphisms, with odds ratio.

Receptor	Polymorphism	Population	Cases	Controls	OR	*p value*	Ref.
P2Y12	H2 haplotype	98	–	–	3.3 (1.1–10.4)	Data not shown	[27]
Glycoprotein Ibα	-5 T/C (major allele)	451	231	220	0.61 (0.4–0.93)	0.03	[37]
Glycoprotein IIb/IIIa	PIA2 (all ages)	139	71	68	2.8 (1.2–6.4)	Data not shown	[45]
	PIA2 (under 60)	78	42	36	6.2 (1.8–22.4)	Data not shown	[45]
Glycoprotein Ia/IIa	807C/T	223	91	132	2.61 (1.26–5.41)	0.009	[58]

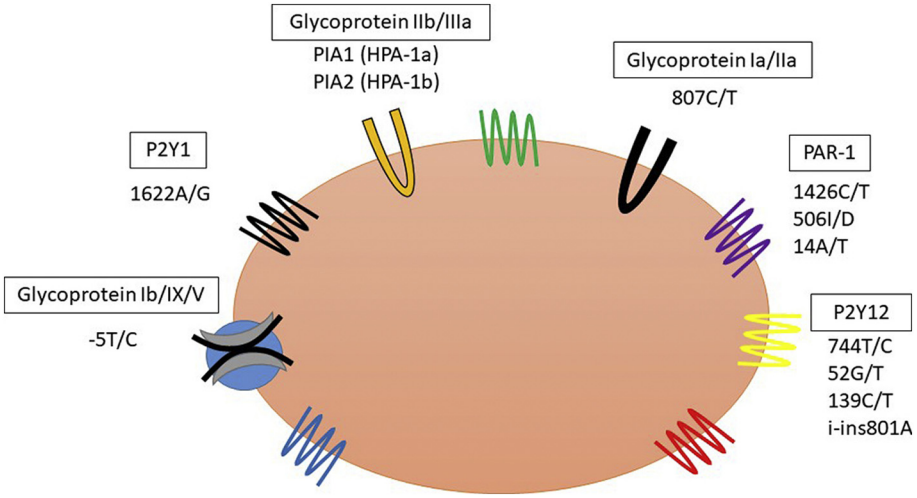

Fig. 3. Distribution of single nucleotide polymorphisms of platelet surface receptors.

Table 4
SNPs listed in the GWAS database and their association with cardiovascular disease.

Receptor	Polymorphism	Gene	Association	*p value*
PAR-1	1426C/T	F2R	Ischaemic Stroke	0.39
			Serum cholesterol	0.46
	506I/D		Lack of association with cardiovascular disease	–
	14A/T		Lack of association with cardiovascular disease	–
P2Y12	744T/C	P2YR12	Lack of association with cardiovascular disease	–
	52G/T		Lack of association with cardiovascular disease	–
P2Y1	1622A/G	P2YR1	Ischaemic stroke	0.056
			Serum cholesterol	0.267
Glycoprotein Ibα	-5T/C	GP1BA	Ischaemic Stroke	0.619
Glycoprotein Ia/IIa	807C/T	ITGA2	Ischaemic Stroke	0.546

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Declaration of competing interests

The authors declare no conflict of interest.

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