Effects of PCSK9 on thrombosis and haemostasis in a variety of metabolic states: Lipids and beyond (Review)

SHAN CHONG[1,2*], GUANGYAN MU[1,3*], XINAN CEN[4], QIAN XIANG[1,3] and YIMIN CUI[1-3]

[1]Institute of Clinical Pharmacology, Peking University First Hospital, Beijing 100191, P.R. China; [2]Department of Pharmacy Administration and Clinical Pharmacy, School of Pharmaceutical Sciences, Peking University Health Science Center, Beijing 100191, P.R. China; [3]Department of Pharmacy, Peking University First Hospital, Beijing 100034, P.R. China; [4]Department of Hematology, Peking University First Hospital, Beijing 100034, P.R. China

Received January 10, 2024; Accepted April 22, 2024

DOI: 10.3892/ijmm.2024.5381

Abstract. Proprotein convertase subtilisin kexin type 9 (PCSK9) inhibitors are widely recognised as being able to induce a potent reduction in low-density lipoprotein-cholesterol. An increasing number of studies have suggested that PCSK9 also influences the haemostatic system by altering platelet function and the coagulation cascade. These findings have significant implications for anti-PCSK9 therapy in patients with specific coagulation conditions, including expanded indications, dose adjustments and drug interactions. The present review summarises the changes in PCSK9 levels in individuals with liver diseases, chronic kidney diseases, diabetes mellitus, cancer and other disease states, and discusses their impact on thrombosis and haemostasis. Furthermore, the structure, effects and regulatory mechanisms of PCSK9 on platelets, coagulation factors, inflammatory cells and endothelial cells during coagulation and haemostasis are described.

Contents

Correspondence to: Professor Qian Xiang or Professor Yimin Cui, Institute of Clinical Pharmacology, Peking University First Hospital, 38 Xueyuan Road, Haidian, Beijing 100191, P.R. China
E-mail: xiangqz@pkufh.com
E-mail: cui.pharm@pkufh.com

*Contributed equally

Key words: proprotein convertase subtilisin kexin type 9, thrombosis, haemostasis, metabolic states

1. Introduction

Proprotein convertase subtilisin kexin type 9 (PCSK9) influences lipid metabolism by accelerating the degradation of low-density lipoprotein (LDL) receptor (LDLR) (1). The clearance of human LDL-cholesterol (LDL-C) depends on the circulation of LDLR in the liver (2). PCSK9, synthesised in hepatocytes, is subsequently secreted and binds to specific LDLRs on hepatocytes, thus forming a trimolecular complex with LDLR and LDL. The PCSK9-LDLR-LDL complexes prevent the recycling of LDLR to the surface of hepatocytes, resulting in elevated plasma levels of LDL-C (3,4). LDL-C is a crucial factor in atherosclerosis and cardiovascular events. In the subendothelial space, LDL-C undergoes oxidation and aggregation, and its small and dense metabolites enter the arterial wall, leading to the progression of atherogenesis and major adverse cardiovascular events (MACEs) (5).

In the early stages, PCSK9 research predominantly centred on abnormal lipid metabolism. In 2000, a locus for autosomal dominant hypercholesterolaemia was found within the *PCSK9* gene (human chromosome 1p33-p34.3) (6), later established as the causative gene for familial hypercholesterolaemia in 2003 (7). Subsequently, naturally occurring *PCSK9* nonsense mutations were detected and associated with significantly low LDL-C levels and the risk of developing cardiovascular disease shortly thereafter. Currently, over a hundred mutations influencing PCSK9 function have been identified (8). Since the launch of the first PCSK9 inhibitor in 2015, unexpected effects have been progressively unveiled. In 2020, patients undergoing treatment with PCSK9 inhibitor were found to exhibit a reduced platelet reactivity and increased sensitivity to aspirin suppression (9). Later that year, it was first confirmed that short-term treatment with evolocumab significantly ameliorated coronary endothelial dysfunction (10). Mechanistic studies on the impact of PCSK9 on thrombosis and inflammation have also garnered attention during this period (please see section 4 below).

Beyond atherosclerotic heart disease, the connection between PCSK9 and heart failure, atrial fibrillation and calcific aortic valve disease has come to light (11). The association between PCSK9 and cardiovascular events appears to transcend lipid levels, yet critical pieces of the puzzle are still missing to explain these findings.

Thrombosis and haemostasis are the leading causes of premature deaths. Apart from increasing plasma levels of LDL-C, PCSK9 regulates the hypercoagulable state and thrombosis processes in disease states. Subsequent studies have underscored this point, indicating that PCSK9 levels in diabetic patients following percutaneous coronary intervention (PCI) were independently associated with MACEs, including thromboembolism risk, but no such association was found in non-diabetic patients (12). The mechanism behind this phenomenon has yet to be elucidated, thereby hindering the tailoring of personalized therapies for patients with concurrent metabolic disorders to achieve further mitigation of residual cardiovascular risk. PCSK9 inhibitors are expected to reach US$ 15,140.3 million by the year 2034, growing at an annual rate of 18.7% (13). The population of patients receiving PCSK9 inhibitors with multiple diagnoses is rapidly increasing. Hence, comprehending the role of PCSK9 in disease states is paramount for enhancing the cost-effectiveness and quality of life for hyperlipidaemia patients globally.

The present review focuses on the effects of changes in PCSK9 levels on thrombosis and haemostasis, and the related modulatory mechanisms of platelet function, coagulation factor activity, inflammatory response and endothelial function in pathological conditions. The aim of the present review was not to comprehensively cover all information regarding the effects of PCSK9 on thrombosis and haemostasis, but rather to focus on disease states, as it was considered that these warrant further extensive research and may have a potential influence on the drug market, regarding the expansion of indications, dose adjustment and drug interactions.

2. PCSK9 structure

PCSK9 is a serine protease consisting of 692 amino acids (14). The structure of PCSK9 reveals three distinct structural domains: The prodomain (aa 32-152), the catalytic domain (aa 153-421) and the C-terminal Cys/His-rich domain (CHRD; aa 453-692) (Fig. 1).

The prodomain is not only essential for the correct folding PCSK9 in the endoplasmic reticulum, but is also related to the lack of activity against exogenous substrates (15). The C-terminus Gln152 of the prodomain binds to His226, filling the oxyanion hole between Asn317 and Ser386 and shielding the active site cleft, thus inhibiting interaction with other proteins and peptides (16). Consequently, the activity of PCSK9 is manifested through its binding to target substrates and escorting the complexes into intracellular degradation compartments, rather than as a protein hydrolase. The prodomain remains in the catalytic groove after self-cleavage, hindering the binding of PCSK9 to other proteins. Removal of the prodomain increases the affinity of PCSK9 for LDLR by 10-fold (17). The PCSK9 Arg93Cys variant is associated with a reduced risk of premature myocardial infarction (18).

The catalytic domain of PCSK9 (aa 153-421) contains essential active sites of PCSK9 and comes into contact with the epidermal growth factor-A (EGF-A) domain of the LDLR, resulting in a ~1,000 $Å^2$ interface (19). As the complex PCSK9-LDLR transitions from extracellular (high pH) to endosomes/lysosomes (low pH), the conformation of PCSK9 due to the pH changes. The difference between the closed and extended conformations revolves around the residue Ser376, which serves as a hinge connecting LDLR. It has been observed that PCSK9 Asp374 forms an extra salt bridge with the EGF-A His306 side chain at a low pH (20). The binding affinity of PCSK9 for LDLR exhibits an increase, from Kd=750±80 nM at pH 7.5 to Kd=8±1 nM at pH 5.5, thus hindering the recycling of LDLR to the cell surface (20). However, the structure of CD36, an important ligand of PCSK9, does not exhibit the EGF-A-like domain, thus the respective binding sequences have yet to be confirmed (14). Variants in the catalytic domain lead to PCSK9 loss or gain of function (21,22).

The hinge region (aa 422-452) links the CHRD. The absence of the CHRD does not affect the binding of PCSK9 to LDLR at a neutral pH (20). However, there is an interaction between CHRD and ligand-binding domain of LDLR at low pH, assisting in the formation of the complex (23). The CHRD comprises three similar modules (M1: aa 453-529, M2: aa 530-603, and M3: aa 604-692), each forming a six-stranded two-sheet β-sandwich. Asn533 in the M2 module is the only glycosylation site in PCSK9, but it is not necessary for PCSK9 activity (16). The three modules exhibit a structural homology to the homotrimeric form of resistin (24). Resistin is an adipocyte-specific hormone associated with hypercoagulative, hypofibrinolytic activities and insulin resistance (25,26).

The *PCSK9* proximal promoter encompasses a functional sterol regulatory element (SRE) that is modulated by intracellular cholesterol levels. SRE binding protein (SREBP) is connected with the SRE of the *PCSK9* and *LDLR* genes, regulating PCSK9 synthesis at the transcriptional level. Following protein cleavage in the Golgi apparatus, mature SREBP-2 enters the nucleus and interacts with the promoters of PCSK9 and LDLR, leading to the increased transcription and translation of both (17,27). The experimental overexpression of SREB-1c stimulates the expression of PCSK9 (28). Another regulator of PCSK9 expression (but not LDLR) is hepatocyte nuclear factor 1 (HNF1). The deficiency or inhibition of HNF1 leads to reduced transcription levels of PCSK9. There is synergy between HNF1 and SRE. Mutations in HNF1 significantly diminish the transactivation activity of SREBP2 on the PCSK9 promote (29). Statins not only deplete intracellular cholesterol, leading to SREBP-2 expression (30,31), but also stimulate hepatic HNF1 expression, thus activating PCSK9 transcription (32).

3. Pleiotropic effects of PCSK9 on cardiovascular disease

Based on the results of several landmark trials of PCSK9 inhibitors (33-35), drugs targeting PCSK9 have swiftly entered the market to improve clinical outcomes by preventing myocardial infarction and coronary revascularization (Table I) (36,37). The enthusiasm for developing PCSK9 inhibitors is still rapidly increasing. A small binding protein of the *PCSK9* gene, lerodalcibep, has been shown to lead to a

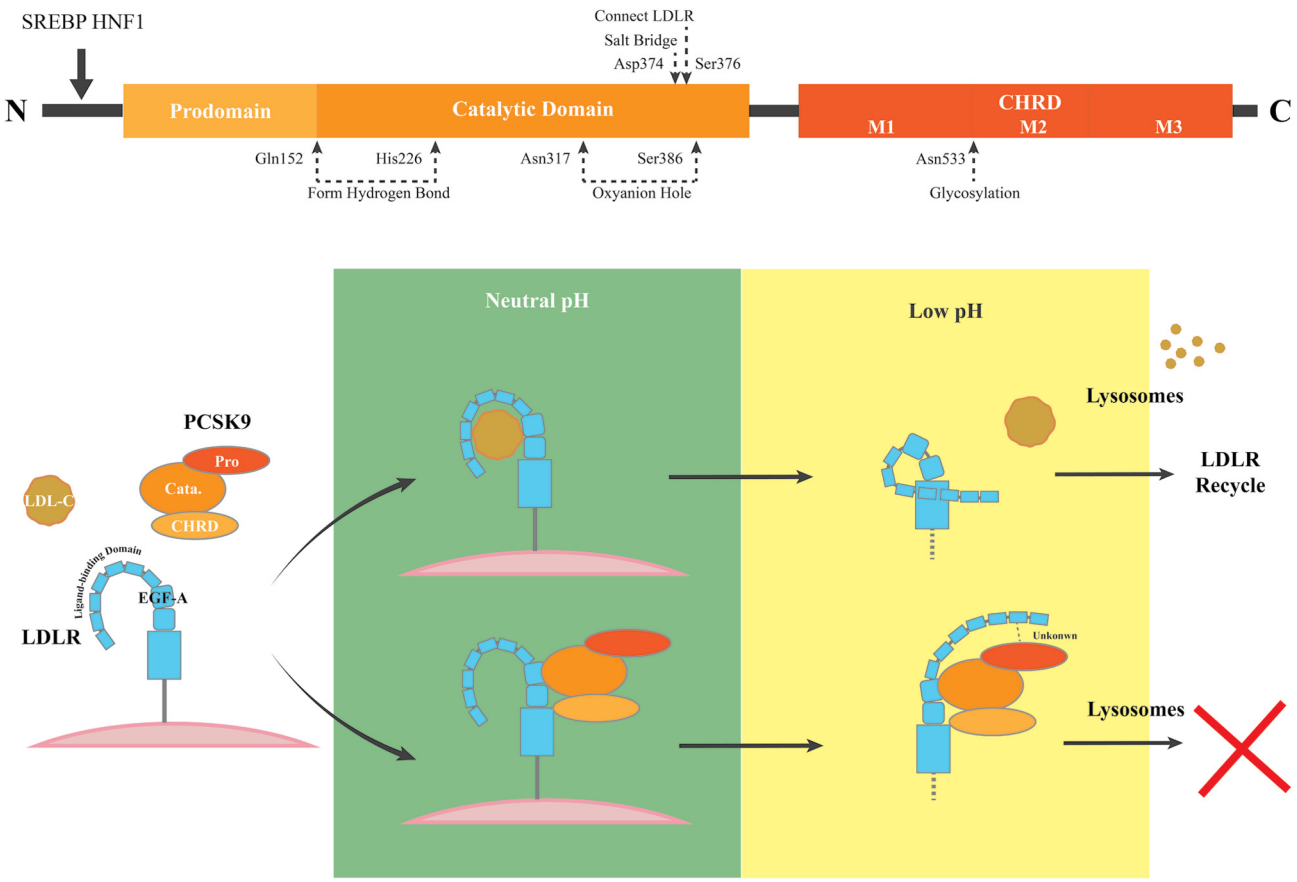

Figure 1. Structure of PCSK9 and the mechanisms of LDLR downregulation. PCSK9, proprotein convertase subtilisin kexin type 9; LDLR, low-density lipoprotein receptor; SREBP, sterol regulatory element-binding protein; HNF1, hepatocyte nuclear factor 1; LDL-C, low-density lipoprotein-cholesterol; Pro, prodomain; Cata., catalytic domain; CHRD, C-terminal Cys/His-rich domain; EGF-A, epidermal growth factor-A.

65% reduction in LDL-C levels in subjects with heterozygous familial hypercholesterolemia at weeks 22 and 24 of phase III clinical trials (38). The oral formulation MK-0616 (an administered macrocyclic peptide), has progressed through phase I clinical trials (39).

In addition to reducing cardiovascular events by decreasing LDL-C in a dose-dependent manner, PCSK9 inhibition exerts a cardiovascular protective effect beyond lipid management. For decades, statins were known as first-line lipid-lowering agents and for the protection of cardiovascular health in hyperlipidaemia. However, the benefits of statins in patients with resistance or intolerance are limited (40). Researchers have observed the plaque stabilization and regression of atherosclerosis using CT angiography and intravascular ultrasound in patients treated with PCSK9 inhibitors (41,42). With the advent of inclisiran, a siRNA PCSK9 inhibitor, appearing on the market, the rate of major adverse cardiovascular events is expected to decrease by as much as 24-30% (43). The effects of PCSK9 on oxidative stress, endothelial injury, inflammation and immunity have been widely described (44-46). The improvement in MACEs due to PCSK9 inhibition may be attributed to various reasons; primarily of interest are the potential direct and indirect roles of PCSK9 in thrombosis and haemostasis.

A high level of PCSK9 has been found to increase platelet reactivity and significantly increase the risk of ischaemic events (47). The results of the Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk (FOURIER) study (34) demonstrated that the incidence of venous thromboembolism (VTE) was decreased in patients treated with PCSK9 inhibitors, which cannot be completely explained by lipid-lowering (48,49). In another study, several platelet activation markers were observed to be significantly reduced after 12 months of PCSK9 monoclonal antibody therapy in patients with hypercholesterolaemia (9), indicating a potential impact of PCSK9 inhibition on platelet function. Additionally, circulating platelets store PCSK9 and release it upon activation, which may promote platelet aggregation and thrombus formation (50).

Abnormal PCSK9 levels are present in several disease states. Patients on maintenance dialysis and following successful renal transplantation have been shown to have higher serum levels of PCSK9 than subjects without chronic kidney disease (51). Patients with liver cirrhosis exhibit lower serum PCSK9 levels, while these levels are increased in patients with hepatitis C virus (HCV)-induced liver cirrhosis (52). PCSK9 levels increase with the severity of breast cancer (53). These specific pathological conditions may lead to changes in thrombosis or haemostasis. Combined with the regulatory role of PCSK9 in thrombosis and the difference in the level of the disease states, thrombosis in metabolism-related diseases may also be regulated by PCSK9, and its regulatory pathway may differ from that in the physiological state.

Table I. US Food and Drug Administration (FDA)-approved PCSK9 inhibitors.

Year	Agent	Mechanism of action	Indication	Mechanism	Specific populations	(Refs.)
2015	Alirocumab	Block interaction with LDLR	· Reduce risk of MI, stroke, coronary revascularization: adults with established cardiovascular disease	· MI, stroke, coronary revascularization: Accumulation and transformation of lipids, inflammatory cells, and necrotic cell debris in the intimal space underneath a monolayer of endothelial cells in the vessel wall, leading to clinical complications	· Renal Impairment: No dose adjustment	(36)
2015	Evolocumab	Block interaction with LDLR	· Reduce LDL-C: Adults with primary hyperlipidemia including HeFH · Reduce LDL-C: Patients (adult, alirocumab; >10 years, evolovumab) with HoFH · Reduce LDL-C: HeFH (>8 years, alirocumab; >10 years, evolovumab)	· Hyperlipidemia: Characterized by elevated cholesterol and triglycerides. Chronic elevated lipid levels promote the development of atherosclerosis. Oxidative stress, mitochondrial dysfunction and apoptosis exerts negative impact on myocardium	· Hepatic Impairment: · No dose adjustment	(10)
2021	Inclisiran	Block mRNA translation	Reduce LDL-C: Adults with primary lipidemia including HeFH	· HoFH and HeFH: A autosomal co-dominant hereditary disorder (*LDLR, APOB, PCSK9*). Characterized by elevated LDL-C, with a 3-13-fold increased risk of atherosclerotic cardiovascular disease		(37)

PCSK9, proprotein convertase subtilisin kexin type 9; LDLR, low-density lipoprotein receptor; MI, myocardial infarction; LDL-C, low-density lipoprotein-cholesterol; HeFH, heterozygous familial hypercholesterolemia; HoFH, homozygous familial hypercholesterolemia; APOB, apolipoprotein B.

4. Mechanisms of PCSK9 in thrombosis and haemostasis

Platelet reactivity, coagulation factor activity and endothelial function are crucial factors affecting thrombosis and haemostasis. Inflammation has been found to contribute to thrombosis mechanisms over the past decade (54). Several studies have proven that PCSK9 has an essential effect on these processes, which may provide a new direction for studying the regulatory mechanisms of thrombosis or bleeding in particular disease states.

PCSK9 and platelets. PCSK9$^{-/-}$ mice have been shown to exhibit slower and less stable thrombus formation compared to PCSK9$^{+/+}$ mice in a model of ferric chloride-induced carotid thrombosis, resulting in incomplete artery occlusion (55). Platelet function is linked to the lipid profile, as cholesterol acquired by platelets modifies the membrane, rendering platelets hypersensitive (46). Previous research has demonstrated that patients with hypercholesterolaemia exhibit elevated membrane cholesterol levels, and treatment with rosuvastatin normalizes the membrane composition, including membrane cholesterol, tissue factor (TF) and TF-dependent procoagulant activity (56). A similar effect between cholesterol and PCSK9 inhibitors appears to be plausible. PCSK9 inhibitors reduce the risk of developing peripheral artery disease following acute coronary syndrome by reducing plasma lipoprotein A [Lp(a)] levels (57,58). However, the exact mechanisms underlying this association are not yet fully understood. Lp(a) possesses anti-fibrinolytic and pro-inflammatory properties, potentially enhancing the occurrence of both venous and arterial thrombosis (59). In addition, Lp(a) contains a Ca^{2+}-independent phospholipase A_2, which serves as a platelet-activating factor and contributes to increased atherogenicity by combining with oxidised phospholipids (60). Apolipoprotein (Apo)B100 is an essential marker for liver cells to recognize LDL-C, while ApoB100 danger-associated signal 1 (ApoBDS-1) is a peptide fragment within ApoB100 (61). Assinger *et al* (61) discovered

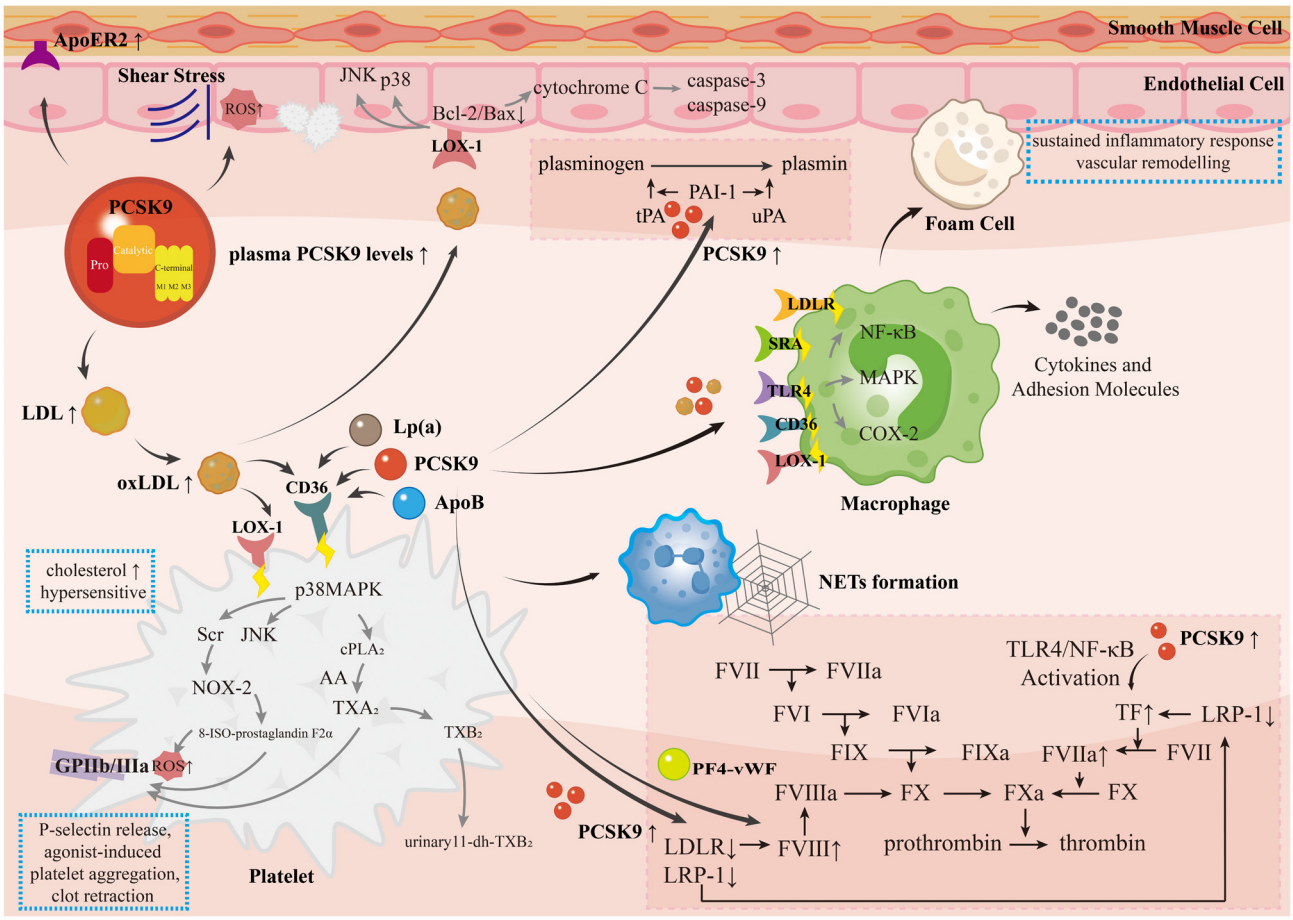

Figure 2. Possible mechanisms of PCSK9 in thrombosis and haemostasis. PCSK9, proprotein convertase subtilisin kexin type 9; LDL, low-density lipoprotein; oxLDL, oxidized low-density lipoprotein; Apo, apolipoprotein; LOX-1, lectin-like oxLDL receptor 1; Lp(a), lipoprotein A; ROS, reactive oxygen species; NOX-2, NAD phosphate oxidase-2; cPLA2, cytosolic phospholipase A2; AA, arachidonic acid; TXA2, thromboxane A2; TXB2, thromboxane B2; PAI-1, plasminogen activator inhibitor type 1; tPA, tissue-type plasminogen activator; uPA, urokinase-type plasminogen activator; LDLR, low-density lipoprotein receptor; SRA, scavenger receptor class A; TLR4, toll-like receptor 4; COX-2, cyclooxygenase 2; LRP, LDLR-related protein; TF, tissue factor; PF4, platelet factor 4.

that ApoBDS-1 activated platelets via p38-mitogen-activated protein kinase (MAPK) and other signalling pathways, contributing to boosting platelet-leukocyte proinflammatory responses. A recent study confirmed that ApoB levels <35 mg/dl reduced the residual risk following acute coronary syndrome, with a greater absolute reduction in MACEs observed in patients with higher baseline levels when treated with alirocumab (62).

Compared to other LDL-C-lowering drugs, where a reduction of 1 mmol/l is associated with a relative risk (RR) of 0.77 [95% confidence interval (CI), 0.75-0.79] for major vascular events, the estimated RR for PCSK9 inhibitors is lower at 0.49 (95% CI, 0.34-0.71) (63). This suggests that although the LDL-C-lowering effect is undoubtedly a key contributor to the haemostasis and thrombosis benefits of PCSK9 inhibition, PCSK9 has also been found to stimulate platelet activation beyond lipids (Fig. 2). First, scavenger receptor CD36, a major platelet glycoprotein, plays a crucial role in dyslipidaemia and the prothrombotic phenotype (64). PCSK9 in the plasma directly combines with CD36 on platelets, thereby enhancing Src kinase phosphorylation. This activation triggers NAD phosphate oxidase-2 (NOX-2), leading to the increased generation of reactive oxygen species (ROS) and the subsequent activation of MAPK-ERK5, ultimately promoting platelet hyperactivity (65,66). Moreover, NOX-2 induces the generation of 8-ISO-prostaglandin F2α, which forms platelet aggregation via GP-IIb/IIIa activation (67). Second, PCSK9 binding to CD36 also activates the JNK and p38 MAPK pathways. The activation of p38 MAPK stimulates cytosolic phospholipase A2, which releases arachidonic acid from phospholipid membranes. Arachidonic acid is then converted into thromboxane A2 (TXA_2) through the cyclooxygenase (COX)-1 and Tx synthase pathways (66). TXA_2 acts synergistically with platelet agonists to enhance platelet aggregation by activating GP-IIb/IIIa receptors. TXA_2 is rapidly metabolised to TXB_2, which can be detected in urine as 11-dehydro-TxB_2 by liver metabolism, indicating platelet activation in patients (68). Third, positive feedback interplay was found between PCSK9 expression and oxidised LDL (oxLDL) generation (69). Elevated levels of oxLDL combined with CD36 and lectin-like oxLDL receptor 1 (LOX-1) follow a pattern similar to that of PCSK9, leading to platelet aggregation and secretion (70). A recent study demonstrated that inclisiran inhibited human umbilical vein endothelial cell death induced by oxLDL, suggesting a potential role in the treatment of atherosclerosis (71). Finally, the aforementioned

mechanism enhances integrin αIIbβ3 activation, P-selectin release, agonist-induced platelet aggregation and clot retraction (65). The PCSK9 regulation of platelets may partly influence the haemostasis process in liver disease.

PCSK9 and coagulation factors. Another mechanism by which the haemostasis process is impacted by PCSK9 involves blood coagulation factors. Coagulation factor VIII (FVIII) is unequivocally a vital participant in the intrinsic pathway, where the lack of FVIII may cause haemophilia (72). Together with LDLR, LDLR-related protein (LRP) modulates FVIII levels (73). Although there are still no experimental results in support of this premise, it is possible that PCSK9 indirectly increases the circulation of FVIII, given its considerable effect on LDLR reduction (73). Additionally, PCSK9 augments plasma FVIII levels *in vivo* by decreasing LRP-1 expression (74). LRP-1 is also associated with TF levels on the cell surface. TF downregulation occurs through rapid internalization when the factor VII (FVII)/TF complex bridges LRP-1 (75). Furthermore, PCSK9 induces the *de novo* synthesis of TF, attributed to the TF/FVIII interaction via the Toll-like receptor 4 (TLR4)/nuclear factor κB (NF-κB) signalling pathway (76), thereby initiating the extrinsic pathway, where abnormal TF levels may contribute to coagulation initiation.

The increased expression of PCSK9 has been reported to contribute to lower a plasma prothrombin time (PT) and to also be associated with a higher risk of MACEs (77). It is known that PT is crucial for exposing the activation of the extrinsic pathway; thus, a low PT suggests that TF and FVII could be in abnormal states (77). It is possible that PCSK9 could promote the formation of the TF-FVII complex.

PCSK9 affects fibrinolysis through plasminogen activator inhibitor-1 (PAI-1). Levine *et al* (78) performed a series of experiments to support this view. RNA sequencing revealed that TM5614, a PAI-1 inhibitor, led to an 86% gene transcript downregulation of PCSK9 in mice. Similar results were also found *in vivo* in mice with hyperlipidaemia. Furthermore, the authors of that study observed that patients with heart failure treated with PCSK9 monoclonal antibodies exhibited a substantial increase in plasma PCSK9 and a reduction in plasma PAI-1 levels (78).

In addition, fibrinogen is also known as coagulation factor I. Candidate gene analysis detected significant associations between total fibrinogen and polymorphisms of PCSK9 and LDLR genes (79). PCSK9 levels were found to be associated with fibrinogen in patients with isolated hyperlipidaemia and stable coronary atherosclerosis (80,81). However, studies on familial hypercholesterolaemia found that PCSK9 inhibitors did not affect fibrinogen and D-dimer levels (82). Conflicting evidence suggests that the association between PCSK9 and fibrinogen should be further investigated for detailed analysis.

PCSK9 and Inflammation. Inflammation plays a cruical role in atherosclerotic plaque and thrombosis. As a result, persistent inflammation is strongly linked to higher risk of ischaemic events (83). Anti-inflammatory therapy can significantly reduce the risk of recurrent cardiovascular events without affecting lipid levels in patients with previous myocardial infarction (84). The PCSK9 pathway promotes the inflammatory response and plays a role in in the thrombosis and atherosclerosis processes (85,86).

PCSK9 is associated with key inflammatory markers, such as white blood cells, fibrinogen and high-sensitivity C-reactive protein (45). However, the underlying mechanisms remain unclear. The increased levels of adhesion molecules and cytokines (e.g., PF4) expressed by endothelial cells under atherosclerosis lead to the attachment of monocytes and lymphocytes at the intima and further stimulate monocytes to differentiate into macrophages (87,88). PF4 is a protein synthesised from platelet particles. Recent research has found that PF4 interacts with von Willebrand factor (VWF) to form immune complexes. The PF4-VWF complex stimulates platelet activation and inflammation, and promotes the formation of immune-associated thrombosis by suppressing a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 in a concentration-dependent manner (89). A cohort study supported the assumption that after 12 months of PCSK9 inhibition therapy, the plasma level of PF4 decreased, which may play a role in cardiovascular event reduction (9). VWF participates in the coagulation process as a carrier and protector of FVIII. Since plasma FVIII levels are increased by PCSK9 (74), it can be hypothesized that low PCSK9 levels may further become a barrier to thrombosis. In summary, the PCSK9-FVIII-PF4 pathway in thrombosis is a direction worthy of further research. This may be helpful to explain VTE reduction in patients with PCSK9 inhibitors. Yurtseven *et al* (90) observed a significant increase in Ly6C (hi) monocytes, which are the inflammatory monocytes, following treatment with PCSK9, followed by an enhancement of their recruitment to the arterial wall. Furthermore, oxLDL and PCSK9 potently bind to LDLR, CD36, LOX-1, TLR4 and scavenger receptor class A on macrophages, thereby stimulating the NF-κB, MAPK and COX-2 pathways. This induction leads to an increased oxLDL uptake by macrophages, as well as to the increased expression of cytokines and mRNA of adhesion molecules, including interleukin (IL)-1β, IL-6, tumour necrosis factor (TNF)-α, chemokine C-X-C motif ligand 2 and monocyte chemotactic protein 1 (MCP1) (70,91,92). In a previous study, compared with the atherosclerotic plaques of untreated ApoE-null mice, mice treated with PCSK9 small hairpin RNA were found to have less macrophage infiltration. The mice in which PCSK9 was suppressed had lower levels of vascular inflammatory regulators, such as TNF-α, IL-1β, MCP1, TLR4 and NF-κB (93). Macrophages turn into foam cells (94). Foam cells, which participate in atherosclerotic plaque rupture, mediate the sustained inflammatory response, resulting in vascular remodelling. After the plaque is disrupted, tissue factor, collagen and other plaque components are exposed, which leads to platelet activation and aggregation, and thrombus formation (95). Clinical research has confirmed that circulating PCSK9 levels are significantly associated with an increased risk of developing myocardial infarction (96).

Neutrophil extracellular traps (NETs) composed of DNA and histones released by neutrophils play a crucial role in trapping bacteria and driving bactericidal activity. However, NETs also play a role in the initiation, formation and propagation of thrombosis. Lipopolysaccharide activates platelets and neutrophils via TLR4, inducing the release of PF4, the expression of

P-selectin and NETosis. The formation of NETs results in the release of TF via autophagy and the activation of FVII (97). An analysis of the inferior vena cava conducted in mice suggested that in comparison with PCSK9-deficient mice, wild-type mice displayed an inferior vena cava thrombus with greater weight, length, myeloid cell recruitment and a greater number of NETs (98). Therefore, PCSK9 promotes thrombosis by affecting leukocyte mobilisation and NET formation. These mechanisms may be of particular importance for patients with diabetes.

PCSK9 and endothelial dysfunction. Vascular endothelial injury is closely associated with thrombosis. Elevated levels of cholesterol expose the vascular walls to oxidative stress, leading to endothelial dysfunction (99). Inflammation induced by endothelial dysfunction abnormally induces platelet attachment to endothelial cells in the arterial walls (100), increasing the cardiovascular risk. Even with short-term treatment, the PCSK9 inhibitors, evolocumab and alirocumab, have been found to improve endothelial function (101,102). Damage to the vascular wall by PCSK9 can occur via a variety of mechanisms.

First, a study on a cohort of 26 patients with cardiovascular disease found that reducing PCSK9 levels significantly increased circulating endothelial progenitor cells, suggesting the promotion of vascular repair (103). Second, the expression of PCSK9 in smooth muscle cells (SMCs) and endothelial cells was found to be highest at proper endothelial shear stress (3-6 dynes/cm^2), and the levels of ROS followed the same pattern as PCSK9 (104). Haemodynamic factors stimulate local lipid accumulation and oxidative stress, accelerating vascular remodelling (105). It has been shown that anti-PCSK9 therapy with alirocumab ameliorates oxidative stress in rats with biliary cirrhosis, without affecting the plasma levels of oxLDL (106). Third, the Bcl-2 protein family and caspases play an essential role in apoptosis. Breaking the balance between the apoptosis inhibitor, B-cell lymphoma-2 (Bcl-2), and the apoptosis inducer, Bcl2-associated X (Bax), can activate caspase-9 and caspase-3 sequentially by regulating the release of cytochrome *c* from the mitochondria to induce cell apoptosis. Studies using human umbilical vein-derived endothelial cells have demonstrated that Bax phosphorylation under oxLDL pressure decreases the Bcl-2/Bax ratio and activates the apoptotic process (107). Following the silencing of PCSK9 with specific small hairpin RNA, the Bcl-2/Bax ratio increases, and the phosphorylation levels of the p38 and JNK signalling pathways are significantly decreased. The programmed death process of endothelial cells is inhibited (108). In addition, PCSK9 affects the metabolism of SMC. It promotes age-related atherosclerosis by downregulating the expression of ApoER2 on SMCs (109). The dysfunction of vascular endothelial cells may be related to abnormal plasma and local PCSK9 levels in patients with and without renal disease undergoing dialysis.

5. Roles of PCSK9 in thrombosis and haemostasis under disease states

Some pathological states may alter the PCSK9 levels, activity and downstream pathways (Fig. 3). Moreover, this may lead to abnormal LDL-C levels and an increased risk of bleeding and thrombosis.

PCSK9 and liver disease. The liver is the predominant synthesis site for multiple proteins involved in coagulation and liver diseases may lead to coagulation disorders presenting as either the impairment or promotion of haemostasis (110). Bleeding was historically considered to be a frequent and fatal complication of liver disease; however, a significant rate of VTE was also observed in patients with chronic liver disease (CLD) (111). The effects of liver disease on haemostasis involve a several mechanisms, and some of the haemostatic changes counteract each other. In patients with liver dysfunction, the reduction of fibrinogen and coagulation factors, such as FII, FV, FVII, FIX, FX and FXI increase the risk of bleeding, while elevated FVIII levels, decreased levels of protein C/S and antithrombin, together with VWF compensation, promote clotting (112). Mild to moderate thrombocytopenia and platelet function defects have been described in patients with CLD, and this can be more severe in those with cirrhosis and splenomegaly (113). However, enhanced *in vivo* platelet activation in patients with liver cirrhosis may contribute to the thrombosis of large vessels (114). There are also abnormalities in the fibrinolytic system in the condition of liver disease, the most prominent of which is the increased level of tissue-type plasminogen activator (tPA) due to reduced hepatic clearance. This may explain the high rate of hyperfibrinolysis (30 to 50%) that occurs in patients with end-stage liver disease (115). However, a causal role of hyperfibrinolysis in bleeding is uncertain due to concomitant changes in haemostasis.

PCSK9 is synthesized mainly in the liver. Impaired hepatic function in patients with liver cirrhosis affects the synthesis and secretion of PCSK9. Feder *et al* (116) reported that patients with liver cirrhosis had lower plasma levels of PCSK9 compared to non-cirrhotic patients. Moreover, the association between serum cholesterol and PCSK9 levels is lacking, which may be due to the overriding effect of PCSK9 on cholesterol metabolism. Lower PCSK9 levels in patients with liver disease are associated with reduced levels of pro-inflammatory factors, suggesting a reduction in the occurrence of atherosclerotic events (117). In a previous study, serum PCSK9 levels and the international normalized ratio were shown to be negatively associated with each other, suggesting a higher risk of bleeding (118). In that study, low systemic levels of PCSK9 were associated with decreased albumin levels and an increased mortality rate in patients suffering from end-stage liver disease.

PCSK9 mediates the degradation of LDLR homologous and non-homologous receptors, including CD36 (119) and LRP1 (120), to regulate inflammation and vascular function. CD36 is a multifunctional signalling molecule inducing inflammation through JNK activation (121). Since CD36 is a key receptor for platelet activation, decreased levels of PCSK9 in patients with liver disease may inhibit CD36 degradation, thereby enhancing the stimulation of the CD36 pathway and promoting platelet aggregation (122). In addition, low levels of PCSK9 have a potential effect on fibrinolysis. tPA is the major intravascular activator of fibrinolysis and is cleared by LRP1 (115). Low PCSK9 levels in liver disease may decrease

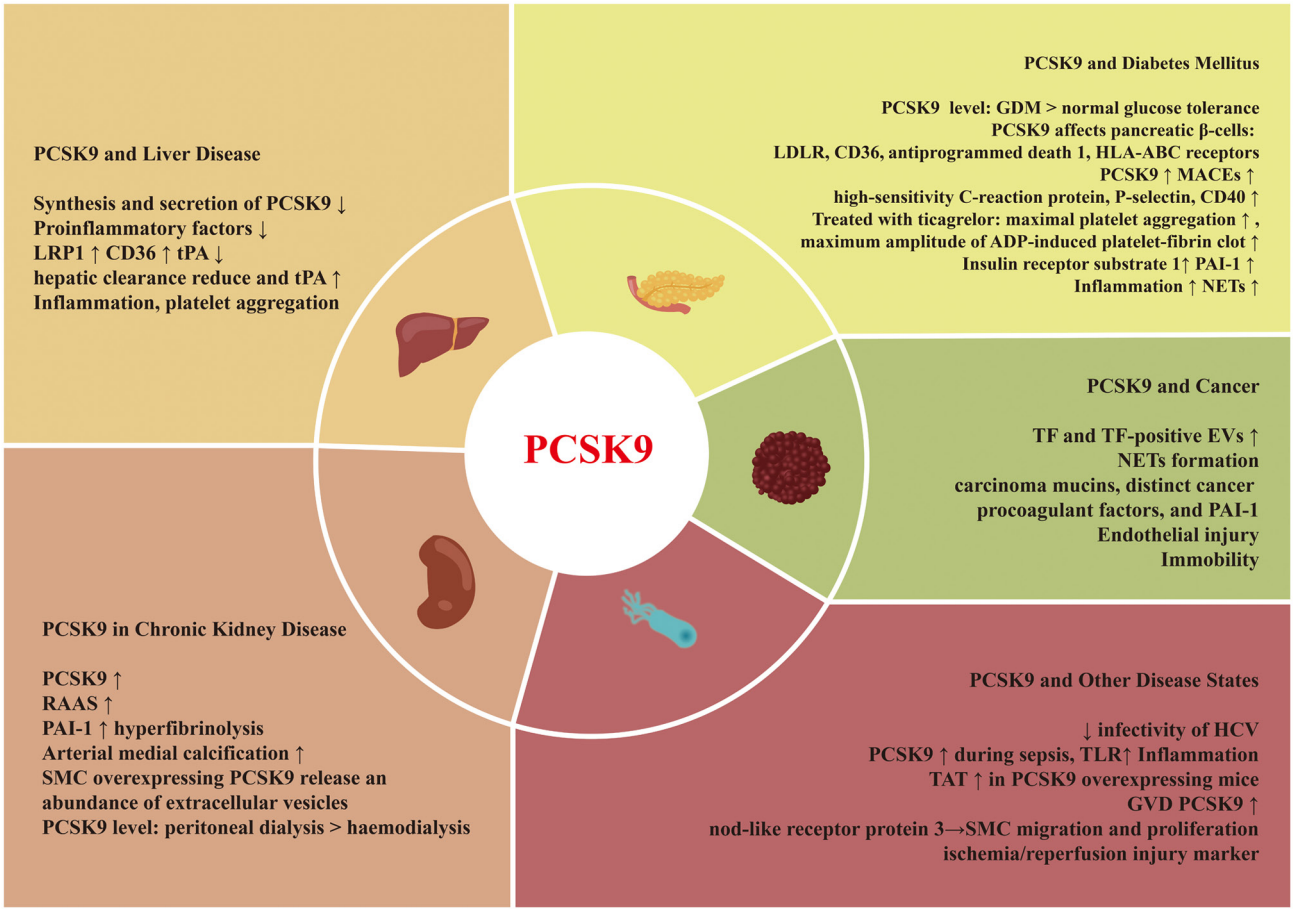

Figure 3. PCSK9 is associated with a variety of disease states with special metabolic characteristics. PCSK9, proprotein convertase subtilisin kexin type 9; LRP1, LDLR-related protein; tPA, tissue-type plasminogen activator; RAAS, renin-angiotensin-aldosterone system; PAI-1, plasminogen activator inhibitor type 1; SMC, smooth muscle cell; GDM, gestational diabetes mellitus; LDLR, low-density lipoprotein receptor; TF, tissue factor; HCV, hepatitis C virus; TLR, Toll-like receptor; TAT, thrombin-antithrombin complex; GVD, graft vascular disease.

LRP1 degradation, thereby promoting LRP1-mediated tPA clearance and alleviating hyperfibrinolysis to a certain extent (120).

PCKS9 and chronic kidney disease. In a previous prospective study involving 5,138 individuals with chronic kidney disease, elevated levels of PCSK9 were shown to be associated with an increased risk of cardiovascular disease (123). Despite an increase in PCSK9 levels in cases of nephrotic syndrome, no association was found with the estimated glomerular filtration rate (124). While some studies have reported an association between PCSK9 and proteinuria, others have revealed opposite results (124,125). PCSK9 is expressed in the kidneys to a lesser extent. The collecting duct is a major source of increased PCSK9 expression in the kidneys, which may be a new driver of hyperlipidaemia in nephrotic syndrome (126).

Chronic kidney disease affects the haemostatic system due to complex alterations in platelets, vessel walls, microparticles and even drugs (127). The changes in PCSK9 levels among patients with nephropathy add a further layer of complexity to haemostasis. The renin-angiotensin-aldosterone system (RAAS) is abnormally active in patients with renal failure, and is associated with increased levels of PAI-1 (127). Given the high level of PCSK9, the interaction between PCSK9 and PAI-1 may promote hyperfibrinolysis (as aforementioned in the sub-section entitled 'PCSK9 and coagulation factors'). Intracellular PCSK9 promotes arterial medial calcification. SMCs overexpressing PCSK9 release an abundance of extracellular vesicles (EVs) that are rich in Ca^{2+} and alkaline phosphatase (128). EVs increase pro-calcific markers, which results in renal impairment by regulating the calcium-phosphorus balance, and eventually promoting calcification of SMCs (129). Arterial medial calcifications promote the progression of atherosclerotic lesions and affect platelet-vessel wall interactions (130).

Zymogen PCSK9 (73 kDa) undergoes processing in the endoplasmic reticulum and Golgi body and is secreted as mature PCSK9 (63 kDa) (131). Dialysis may influence PCSK9 kinetics, although available literature on this topic is limited. A prevoius small cohort study indicated increased PCSK9 levels in patients receiving peritoneal dialysis as compared to those on haemodialysis and in control subjects (124). Notably, abnormal platelet activation occurs in patients treated with peritoneal dialysis (127). Therefore, the potential benefits of anti-PCSK9 therapy in these patients are worthy of investigation.

PCSK9 and diabetes mellitus. Cardiovascular disease tends to develop in patients with either type 1 (T1DM) or type 2 (T2DM) diabetes mellitus. The lipid profile of patients

with well-controlled T1DM is similar to that of the general population (132). However, patients with T2DM frequently exhibit elevated levels of triglycerides and non-high-density lipoprotein-cholesterol, even with good glycaemic control (133). Patients with T2DM are considered to be in a hypercoagulable state induced by insulin resistance and endothelial dysfunction, which causes microvascular and macrovascular complications by augmenting atherosclerosis (134). Due to the increased risk of MACEs, clinical guidelines the stricter lipid targets for individuals with diabetes (135). Hyperglycaemia leads to platelet hyperactivity through various mechanisms, including the glycation of platelet surface proteins, the expression of GPIIb and P-selectin, and PKC activation (136). Moreover, T2DM is associated with chronic inflammatory disorders (137). Neutrophils isolated from diabetic individuals are more prone to forming NETs, and the interaction between NETs and macrophages contributes to persistent macrophage inflammation (138). Hypofibrinolysis is also widely observed in T2DM, partly due to the elevated concentration and activity of PAI-1 (139). Platelet hyperactivity, fibrinolytic inhibition, oxidative stress and inflammation further promote thrombosis (136).

Circulating levels of PCSK9 are significantly higher in pregnant women with gestational diabetes mellitus (GDM) compared to those with a normal glucose tolerance. PCSK9 level is positively associated with fasting plasma glucose, glycosylated haemoglobin, total cholesterol, and LDL-C levels in patients with GDM (140). PCSK9 deficiency leads to an increased expression of LDLR in pancreatic β-cells (141). PCSK9 knockdown also promotes intracellular cholesterol accumulation, islet function and insulin secretion (141,142). Specifically, PCSK9 interacts with human pancreatic β-cells through LDLR and an intracellular signalling mechanism that regulates the expression of CD36, anti-programmed death 1 and HLA-ABC receptors (141). It remains unclear whether the use of PCSK9 inhibitors increases the risk of developing diabetes (143,144). Considering the inconsistent results, further investigations are required to elucidate the association between PCSK9 levels and diabetes.

PCSK9 monoclonal antibody therapy is safe and effective for patients with diabetes (145). Plasma PCSK9 levels appear to affect inflammation and coagulation in patients with diabetes mellitus. A previous observational study reported that patients with diabetes and PCSK9 levels of ≤43.5 ng/ml had the highest survival from MACEs (12). High PCSK9 levels were found to be associated with MACEs in diabetic patients undergoing primary PCI, possibly due to their strong association with inflammation and platelet activation markers, such as high-sensitivity C-reactive protein, P-selectin and CD40L (12). In diabetic patients treated with ticagrelor, PCSK9 levels were positively associated with maximal platelet aggregation and maximum amplitude of ADP-induced platelet-fibrin clots, while no association was found in non-diabetic patients (12). In addition, PCSK9 levels have been shown to be independently associated with the platelet count in patients with T2DM (146).

It appears that patients with DM experience additional anticoagulation benefits from PCSK9 inhibitors. On the one hand, diabetes is associated with chronic low-grade inflammation, suggesting that the inhibition of PCSK9 in diabetic patients may yield greater cardiovascular benefits through its anti-inflammatory effects than in non-diabetic patients. On the other hand, PCSK9 may also interact with fibrinolytic processes. Patients with T2DM exhibit impaired fibrinolysis due to the downregulation of insulin receptor substrate 1 in endothelial cells. The level of PAI-1 is increased by 25-280% compared to that in non-diabetic patients (139). Since PCSK9 levels positively correlate with PAI-1 levels (78), diabetic patients are more likely to have high PCSK9 levels and hypercoagulable states. Although there is a lack of evidence on the effect of PCSK9 levels on the fibrinolytic state, the role of PCSK9 in the regulation of the fibrinolytic process should be further explored.

PCSK9 and cancer. Thromboembolism is the second leading cause of mortality among patients with cancer undergoing outpatient chemotherapy, immediately following the progression of cancer (147). To the best of our knowledge, to date, no large-scale clinical studies have evaluated the effects of anti-PCSK9 therapy on thrombosis in patients with cancer, although it is likely a promising research area. It is well-known that hypercoagulability, endothelial injury and abnormal haemodynamics contribute to thrombosis, and cancer-associated thrombosis (CAT) presents peculiarities in pathophysiological mechanisms. First, host and malignant cells abnormally synthesize excessive TF and TF-positive EVs, which initiate the extrinsic pathway (148). TF-positive EVs generated from endothelial cells, monocytes and macrophages play a role in thrombosis, while platelet-derived TF and TF-positive EVs are controversial. The exposed phosphatidylserine on TF-positive EVs appears to promote the binding of coagulation factors, rather than TF. NETs serve as a scaffold for various procoagulants and act as mediators of CAT (149). It has been shown that 4T1-derived exosome range EVs induce NET formation in the neutrophils of granulocyte colony-stimulating factor-treated mice (150). Human studies have shown that blood and tissue samples from patients with gastric cancer were more likely to form NETs, promoting the conversion of thrombin and fibrin to develop thrombosis compared with healthy individuals (151). Moreover, other substances produced by cancer cells also exacerbate the hypercoagulable state, such as carcinoma mucins, distinct cancer procoagulant factors and PAI-1 (152). Second, surgical procedures, catheters and chemotherapy often lead to endothelial injury (153). Third, oncologic pain causes immobility, which aggravates circulatory stasis (154).

PCSK9 and other disease states. Changes in immune-related factors can lead to alterations in PCSK9 levels and functions, which can also affect clotting functions. Herein, the changes in the levels of PCSK9 in infection and transplantation are described. Research on the marine fish, *Epinephelus coioides*, has demonstrated that the upregulation of PCSK9 expression increased the activities of NF-κB promoter, Singapore grouper iridovirus-induced apoptosis, and pro-inflammatory factors (155). These findings highlight the role of PCSK9 in modulating innate immunity in pathogen infections. Moreover, PCSK9 reduces the infectivity of HCV by acting on LDLR, very LDLR, scavenger receptor class B type 1 and CD81 receptors on hepatocytes (156). PCSK9 expression is upregulated during sepsis, increasing cholesterol levels and promoting

cholesterol accumulation in immune cells (157). These factors improve TLR function and promote inflammation (157,158). Thrombin-antithrombin complex levels have been found to be elevated in PCSK9-overexpressing mice, which indicates that PCSK9 exacerbates the hypercoagulation state in early sepsis (159). Therefore, although PCSK9 promotes pathogen clearance, it is possible that PCSK9 also exacerbates the procoagulant state of the body. A number of organ transplant patients suffer from graft vascular disease (GVD). Previous studies have reported that PCSK9 levels are upregulated in wild-type GVD mice, while PCSK9 knockout mice have a reduced vascular stenosis, intimal hyperplasia area and collagen deposition (160). Furthermore, the lack of PCSK9 probably inhibits the inflammasome through the nod-like receptor protein 3 pathway and suppresses the migration and proliferation of vascular SMCs (161). Previous studies on patients who have undergone a heart transplant suggested that PCSK9 inhibitors can stabilise coronary intimal hyperplasia (161). Additionally, donor-specific antibodies do not increase after 1 month of treatment (162). PCSK9 is a potential marker of ischemia/reperfusion injury during visceral vascular surgery or myocardial infarction (163). Ischemia/reperfusion triggers the upregulation of PCSK9 and increased the levels of autophagy regulatory proteins light chain 3 and beclin-1, at the same time, activating the Bcl-2/adenovirus E1B 19-kDa interacting protein pathway aggravated ischemia/reperfusion injury (164).

6. Conclusion and future prospects

Based on their superior safety profile, current PCSK9 inhibitors have been approved for use in the majority of patients with hyperlipidaemia, including those with liver disease, chronic kidney disease, diabetes and cancer. Nevertheless, both clinical evidence and *in vitro* data suggest that during pathological conditions, the concentration and effects of PCSK9 could differ from those in general patients under pathological conditions. The effect of PCSK9 on thrombosis and haemostasis could also be intricate. Generally, PCSK9 levels are elevated in several metabolic states, primarily inciting thrombophilia through platelet activation and inflammation pathways. Nonetheless, in certain cases, reduced levels of PCSK9 may alleviate hyperfibrinolysis by mediating the PAI-1 pathway.

To date, numerous key questions remain to be addressed. The PAI-1/PCSK9 pathways regulate the fibrinolysis process; however, the specific mechanism that dominates fibrinolysis in patients with liver damage remains to be determined. It also remains unclear whether the concurrent inhibition of the RAAS and PCSK9 inhibitors may be beneficial in patients with chronic kidney disease. Future research is thus required to elucidate the following: i) The mechanisms through which different disease states alter PCSK9 function and its impact on the clotting process beyond serum concentrations; ii) the specific roles of PCSK9 in local coagulation within particular tissues; and iii) provide further evidence of the extent of the inhibitory effects of PCSK9 on haemostasis and thrombosis, and determine the associated clinical outcomes. Therefore, the use of anti-PCSK9 therapy in specific patients may alter the course of haemostasis and thrombosis, presenting a double-edged sword. On the one hand, the incidence of bleeding events may be increased, while on the other hand, patients with thrombotic tendencies may benefit. At the same time, drug interactions can be reshuffled. It is expected that more high-quality evidence will emerge, as it may change medication regimens for certain patients.

Acknowledgements

The authors would like to thank Professor Yan Zhang (Department of Cardiology, Peking University First Hospital) for his professional guidance of this review during the revision process.

Funding

The present study was supported by the National Key Research and Development Program of China (grant nos. 2021YFC2500500 and 2021YFC2500503), the National High Level Hospital Clinical Research Funding (Scientific Research Fund of Peking University First Hospital (grant no. 2022SF15), and the National Natural Science Foundation of China General Program (grant nos. 82073935 and 82373950).

Availability of data and materials

Not applicable.

Authors' contributions

SC, GM and QX were involved in the investigative aspects of the review, such as the literature search for related studies. SC, GM and XC were involved in visualization (preparation of the figures). SC and GM were involved in the writing of the original draft of the manuscript. XC, QX and YC were involved in the writing, reviewing and editing of the manuscript. GM, QX and YC were involved in the conceptualization and supervision of the study, and in funding acquisition. All authors have read and agreed to the published version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Mu G, Xiang Q, Zhou S, Liu Z, Qi L, Jiang J, Gong Y, Xie Q, Wang Z, Zhang H, *et al*: Efficacy and safety of PCSK9 monoclonal antibodies in patients at high cardiovascular risk: An updated systematic review and meta-analysis of 32 randomized controlled trial. Adv Ther 37: 1496-1521, 2020.
2. Zhang DW, Garuti R, Tang WJ, Cohen JC and Hobbs HH: Structural requirements for PCSK9-mediated degradation of the low-density lipoprotein receptor. Proc Natl Acad Sci USA 105: 13045-13050, 2008.

3. Paciullo F, Momi S and Gresele P: PCSK9 in haemostasis and thrombosis: Possible pleiotropic effects of PCSK9 inhibitors in cardiovascular prevention. Thromb Haemost 119: 359-367, 2019.
4. Grundy SM, Stone NJ, Bailey AL, Beam C, Birtcher KK, Blumenthal RS, Braun LT, de Ferranti S, Faiella-Tommasino J, Forman DE, *et al*: 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA guideline on the management of blood cholesterol: Executive summary: A report of the American College of Cardiology/American Heart Association Task Force on clinical practice guidelines. J Am Coll Cardiol 73: 3168-3209, 2019.
5. Henein MY, Vancheri S, Longo G and Vancheri F: The role of inflammation in cardiovascular disease. Int J Mol Sci 23: 12906, 2022.
6. Hunt SC, Hopkins PN, Bulka K, McDermott MT, Thorne TL, Wardell BB, Bowen BR, Ballinger DG, Skolnick MH, Samuels ME, *et al*: Genetic localization to chromosome 1p32 of the third locus for familial hypercholesterolemia in a Utah kindred. Arterioscler Thromb Vasc Biol 20: 1089-1093, 2000.
7. Abifadel M, Varret M, Rabès JP, Allard D, Ouguerram K, Devillers M, Cruaud C, Benjannet S, Wickham L, Erlich D, *et al*: Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. Nat Genet 34: 154-156, 2003.
8. Hummelgaard S, Vilstrup JP, Gustafsen C, Glerup S and Weyer K: Targeting PCSK9 to tackle cardiovascular disease. Pharmacol Ther 249: 108480, 2023.
9. Barale C, Bonomo K, Frascaroli C, Morotti A, Guerrasio A, Cavalot F and Russo I: Platelet function and activation markers in primary hypercholesterolemia treated with anti-PCSK9 monoclonal antibody: A 12-month follow-up. Nutr Metab Cardiovasc Dis 30: 282-291, 2020.
10. Andreadou I, Schulz R, Badimon L, Adameová A, Kleinbongard P, Lecour S, Nikolaou PE, Falcão-Pires I, Vilahur G, Woudberg N, *et al*: Hyperlipidaemia and cardioprotection: Animal models for translational studies. Br J Pharmacol 177: 5287-5311, 2020.
11. Guo Y, Yan B, Tai S, Zhou S and Zheng XL: CSK9: Associated with cardiac diseases and their risk factors? Arch Biochem Biophys 704: 108717, 2021.
12. Song L, Zhao X, Chen R, Li J, Zhou J, Liu C, Zhou P, Wang Y, Chen Y, Zhao H and Yan H: Association of PCSK9 with inflammation and platelet activation markers and recurrent car-diovascular risks in STEMI patients undergoing primary PCI with or without diabetes. Cardiovasc Diabetol 21: 80, 2022.
13. INC., F.M.I: PCSK9 inhibitor market outlook from 2024 to 2034. 2023 (cited 2023 March 31st); Available from: https://www.futuremarketinsights.com/reports/pcsk9-inhibitors-market.
14. Seidah NG, Awan Z, Chrétien M and Mbikay M: PCSK9: A key modulator of cardiovascular health. Circ Res 114: 1022-1036, 2014.
15. Garvie CW, Fraley CV, Elowe NH, Culyba EK, Lemke CT, Hubbard BK, Kaushik VK and Daniels DS: Point mutations at the catalytic site of PCSK9 inhibit folding, autoprocessing, and interaction with the LDL receptor. Protein Sci 25: 2018-2027, 2016.
16. Cunningham D, Danley DE, Geoghegan KF, Griffor MC, Hawkins JL, Subashi TA, Varghese AH, Ammirati MJ, Culp JS, Hoth LR, *et al*: Structural and biophysical studies of PCSK9 and its mutants linked to familial hypercholesterolemia. Nat Struct Mol Biol 14: 413-419, 2007.
17. Wiciński M, Żak J, Malinowski B, Popek G and Grześk G: PCSK9 signaling pathways and their potential importance in clinical practice. EPMA J 8: 391-402, 2017.
18. Yang L, Pu T, Zhang Y, Yan H, Yu H and Gao W: The R93C variant of PCSK9 reduces the risk of premature mi in a Chinese Han population. Front Genet 13: 875269, 2022.
19. Kwon HJ, Lagace TA, McNutt MC, Horton JD and Deisenhofer J: Molecular basis for LDL receptor recognition by PCSK9. Proc Natl Acad Sci USA 105: 1820-1825, 2008.
20. Lo Surdo P, Bottomley MJ, Calzetta A, Settembre EC, Cirillo A, Pandit S, Ni YG, Hubbard B, Sitlani A and Carfí A: Mechanistic implications for LDL receptor degradation from the PCSK9/LDLR structure at neutral pH. EMBO Rep 12: 1300-1305, 2011.
21. Cameron J, Holla OL, Laerdahl JK, Kulseth MA, Ranheim T, Rognes T, Berge KE and Leren TP: Characterization of novel mutations in the catalytic domain of the PCSK9 gene. J Intern Med 263: 420-431, 2008.
22. Timms KM, Wagner S, Samuels ME, Forbey K, Goldfine H, Jammulapati S, Skolnick MH, Hopkins PN, Hunt SC and Shattuck DM: A mutation in PCSK9 causing autosomal-dominant hypercholesterolemia in a Utah pedigree. Hum Genet 114: 349-353, 2004.
23. Yamamoto T, Lu C and Ryan R: A Two-step binding model of PCSK9 interaction with the low density lipoprotein receptor. J Biol Chem 286: 5464-5470, 2011.
24. Hampton EN, Knuth MW, Li J, Harris JL, Lesley SA and Spraggon G: The self-inhibited structure of full-length PCSK9 at 1.9 A reveals structural homology with resistin within the C-terminal domain. Proc Natl Acad Sci USA 104: 14604-14609, 2007.
25. Fang WQ, Zhang Q, Peng YB, Chen M, Lin XP, Wu JH, Cai CH, Mei YF and Jin H: Resistin level is positively correlated with thrombotic complications in Southern Chinese metabolic syndrome patients. J Endocrinol Invest 34: e36-e42, 2011.
26. Jamaluddin MS, Weakley SM, Yao Q and Chen C: Resistin: Functional roles and therapeutic considerations for cardiovascular disease. Br J Pharmacol 165: 622-632, 2012.
27. Jeong HJ, Lee HS, Kim KS, Kim YK, Yoon D and Park SW: Sterol-dependent regulation of proprotein Convertase Subtilisin/Kexin type 9 expression by sterol-regulatory element binding protein-2. J Lipid Res 49: 399-409, 2008.
28. Costet P, Cariou B, Lambert G, Lalanne F, Lardeux B, Jarnoux AL, Grefhorst A, Staels B and Krempf M: Hepatic PCSK9 expression is regulated by nutritional status via insulin and sterol regulatory element-binding protein 1c. J Biol Chem 281: 6211-6218, 2006.
29. Li H, Dong B, Park SW, Lee HS, Chen W and Liu J: Hepatocyte nuclear factor 1alpha plays a critical role in PCSK9 gene transcription and regulation by the natural hypocholesterolemic compound berberine. J Biol Chem 284: 28885-28895, 2009.
30. Raal F, Panz V, Immelman A and Pilcher G: Elevated PCSK9 levels in untreated patients with heterozygous or homozygous familial hypercholesterolemia and the response to high-dose statin therapy. J Am Heart Assoc 2: e000028, 2013.
31. Zhang L, McCabe T, Condra JH, Ni YG, Peterson LB, Wang W, Strack AM, Wang F, Pandit S, Hammond H, *et al*: An anti-PCSK9 antibody reduces LDL-cholesterol on top of a statin and suppresses hepatocyte SREBP-regulated genes. Int J Biol Sci 8: 310-327, 2012.
32. Dong B, Singh AB, Shende VR and Liu J: Hepatic HNF1 transcription factors control the induction of PCSK9 mediated by rosuvastatin in normolipidemic hamsters. Int J Mol Med 39: 749-756, 2017.
33. Robinson JG, Farnier M, Krempf M, Bergeron J, Luc G, Averna M, Stroes ES, Langslet G, Raal FJ, El Shahawy M, *et al*: Efficacy and safety of alirocumab in reducing lipids and cardiovascular events. N Engl J Med 372: 1489-1499, 2015.
34. Sabatine MS, Giugliano RP, Keech AC, Honarpour N, Wiviott SD, Murphy SA, Kuder JF, Wang H, Liu T, Wasserman SM, *et al*: Evolocumab and clinical outcomes in patients with cardiovascular disease. N Engl J Med 376: 1713-1722, 2017.
35. Ray KK, Wright RS, Kallend D, Koenig W, Leiter LA, Raal FJ, Bisch JA, Richardson T, Jaros M, Wijngaard PLJ, *et al*: Two phase 3 trials of inclisiran in patients with elevated LDL cholesterol. N Engl J Med 382: 1507-1519, 2020.
36. Björkegren JLM and Lusis AJ: Atherosclerosis: Recent developments. Cell 185: 1630-1645, 2022.
37. Santos RD, Gidding SS, Hegele RA, Cuchel MA, Barter PJ, Watts GF, Baum SJ, Catapano AL, Chapman MJ, Defesche JC, *et al*: Defining severe familial hypercholesterolaemia and the implications for clinical management: A consensus statement from the international atherosclerosis society severe familial hypercholesterolemia panel. Lancet Diabetes Endocrinol 4: 850-861, 2016.
38. Raal F, Fourie N, Scott R, Blom D, De Vries Basson M, Kayikcioglu M, Caldwell K, Kallend D and Stein E; LIBerate-HeFH Investigators: Long-term efficacy and safety of lerodalcibep in heterozygous familial hypercholesterolaemia: The LIBerate-HeFH trial. Eur Heart J 44: 4272-4280, 2023.
39. Agarwala A, Asim R and Ballantyne CM: Oral PCSK9 inhibitors. Curr Atheroscler Rep: Mar 27, 2024 doi: 10.1007/s11883-024-01199-2 (Epub ahead of print).
40. Gallego-Colon E, Daum A and Yosefy C: Statins and PCSK9 inhibitors: A new lipid-lowering therapy. Eur J Pharmacol 878: 173114, 2020.

41. Baumann S, Kettel L, Stach K, Özdemir GH, Renker M, Tesche C, Becher T, Hetjens S, Schoepf UJ, Akin I, *et al*: Serial changes in coronary plaque formation using CT angiography in patients undergoing PCSK9-Inhibitor therapy with 1-year Follow-up. J Thorac Imaging 37: 285-291, 2022.
42. Ota H, Omori H, Kawasaki M, Hirakawa A and Matsuo H: Clinical impact of PCSK9 inhibitor on stabilization and regression of lipid-rich coronary plaques: A near-infrared spectroscopy study. Eur Heart J Cardiovasc Imaging 23: 217-228, 2022.
43. Nishikido T: Clinical potential of inclisiran for patients with a high risk of atherosclerotic cardiovascular disease. Cardiovasc Diabetol 22: 20, 2023.
44. Cammisotto V, Baratta F, Simeone PG, Barale C, Lupia E, Galardo G, Santilli F, Russo I and Pignatelli P: Proprotein convertase subtilisin Kexin type 9 (PCSK9) beyond lipids: The role in oxidative stress and thrombosis. Antioxidants (Basel) 11: 569, 2022.
45. Momtazi-Borojeni AA, Sabouri-Rad S, Gotto AM, Pirro M, Banach M, Awan Z, Barreto GE and Sahebkar A: PCSK9 and inflammation: A review of experimental and clinical evidence. Eur Heart J Cardiovasc Pharmacother 5: 237-245, 2019.
46. Luquero A, Badimon L and Borrell-Pages M: PCSK9 functions in atherosclerosis are not limited to plasmatic LDL-Cholesterol regulation. Front Cardiovasc Med 8: 639727, 2021.
47. Navarese EP, Kolodziejczak M, Winter MP, Alimohammadi A, Lang IM, Buffon A, Lip GY and Siller-Matula JM: Association of PCSK9 with platelet reactivity in patients with acute coronary syndrome treated with prasugrel or ticagrelor: The PCSK9-REACT study. Int J Cardiol 227: 644-649, 2017.
48. Marston NA, Gurmu Y, Melloni GEM, Bonaca M, Gencer B, Sever PS, Pedersen TR, Keech AC, Roselli C, Lubitz SA, *et al*: The effect of PCSK9 (Proprotein Convertase Subtilisin/Kexin type 9) inhibition on the risk of venous thromboembolism. Circulation 141: 1600-1607, 2020.
49. Zaccardi F, Kunutsor SK, Seidu S, Davies MJ and Khunti K: Is the lower risk of venous thromboembolism with statins related to low-density-lipoprotein reduction? A network meta-analysis and meta-regression of randomised controlled trials. Atherosclerosis 271: 223-231, 2018.
50. Petersen-Uribe Á, Kremser M, Rohlfing AK, Castor T, Kolb K, Dicenta V, Emschermann F, Li B, Borst O, Rath D, *et al*: Platelet-derived PCSK9 is associated with LDL metabolism and modulates atherothrombotic mechanisms in coronary artery disease. Int J Mol Sci 22: 11179, 2021.
51. Konarzewski M, Szolkiewicz M, Sucajtys-Szulc E, Blaszak J, Lizakowski S, Swierczynski J and Rutkowski B: Elevated circulating PCSK-9 concentration in renal failure patients is corrected by renal replacement therapy. Am J Nephrol 40: 157-163, 2014.
52. Grimm J, Peschel G, Müller M, Schacherer D, Wiest R, Weigand K and Buechler C: Rapid decline of serum proprotein convertase Subtilisin/Kexin 9 (PCSK9) in Non-cirrhotic patients with chronic Hepatitis C infection receiving direct-acting antiviral therapy. J Clin Med 10: 1621, 2021.
53. Wong Chong E, Joncas FH, Seidah NG, Calon F, Diorio C and Gangloff A: Circulating levels of PCSK9, ANGPTL3 and Lp(a) in stage III breast cancers. BMC Cancer 22: 1049, 2022.
54. Stark K and Massberg S: Interplay between inflammation and thrombosis in cardiovascular pathology. Nat Rev Cardiol 18: 666-682, 2021.
55. Camera M, Rossetti L, Barbieri SS, Zanotti I, Canciani B, Trabattoni D, Ruscica M, Tremoli E and Ferri N: PCSK9 as a positive modulator of platelet activation. J Am Coll Cardiol 71: 952-954, 2018.
56. Panes O, González C, Hidalgo P, Valderas JP, Acevedo M, Contreras S, Sánchez X, Pereira J, Rigotti A and Mezzano D: Platelet tissue factor activity and membrane cholesterol are increased in hypercholesterolemia and normalized by rosuvastatin, but not by atorvastatin. Atherosclerosis 257: 164-171, 2017.
57. Schwartz GG, Szarek M, Bittner VA, Diaz R, Goodman SG, Jukema JW, Landmesser U, López-Jaramillo P, Manvelian G, Pordy R, *et al*: Lipoprotein(a) and benefit of PCSK9 inhibition in patients with nominally controlled LDL cholesterol. J Am Coll Cardiol 78: 421-433, 2021.
58. Schwartz GG, Steg PG, Szarek M, Bittner VA, Diaz R, Goodman SG, Kim YU, Jukema JW, Pordy R, Roe MT, *et al*: Peripheral artery disease and venous thromboembolic events after acute coronary syndrome: Role of Lipoprotein(a) and modification by Alirocumab: Prespecified analysis of the ODYSSEY OUTCOMES randomized clinical trial. Circulation 141: 1608-1617, 2020.
59. Boffa MB: Beyond fibrinolysis: The confounding role of Lp(a) in thrombosis. Atherosclerosis 349: 72-81, 2022.
60. Tsimikas S, Tsironis LD and Tselepis AD: New insights into the role of lipoprotein(a)-associated lipoprotein-associated phospholipase A2 in atherosclerosis and cardiovascular disease. Arterioscler Thromb Vasc Biol 27: 2094-2099, 2007.
61. Assinger A, Wang Y, Butler LM, Hansson GK, Yan ZQ, Söderberg-Nauclér C and Ketelhuth DF: Apolipoprotein B100 danger-associated signal 1 (ApoBDS-1) triggers platelet activation and boosts platelet-leukocyte proinflammatory responses. Thromb Haemost 112: 332-341, 2014.
62. Hagström E, Steg PG, Szarek M, Bhatt DL, Bittner VA, Danchin N, Diaz R, Goodman SG, Harrington RA, Jukema JW, *et al*: Apolipoprotein B, residual cardiovascular risk after acute coronary syndrome, and effects of alirocumab. Circulation 146: 657-672, 2022.
63. Silverman MG, Ference BA, Im K, Wiviott SD, Giugliano RP, Grundy SM, Braunwald E and Sabatine MS: Association between lowering LDL-C and cardiovascular risk reduction among different therapeutic interventions: A systematic review and Meta-analysis. JAMA 316: 1289-1297, 2016.
64. Podrez EA, Byzova TV, Febbraio M, Salomon RG, Ma Y, Valiyaveettil M, Poliakov E, Sun M, Finton PJ, Curtis BR, *et al*: Platelet CD36 links hyperlipidemia, oxidant stress and a prothrombotic phenotype. Nat Med 13: 1086-1095, 2007.
65. Magwenzi S, Woodward C, Wraith KS, Aburima A, Raslan Z, Jones H, McNeil C, Wheatcroft S, Yuldasheva N, Febbriao M, *et al*: Oxidized LDL activates blood platelets through CD36/NOX2-mediated inhibition of the cGMP/protein kinase G signaling cascade. Blood 125: 2693-2703, 2015.
66. Qi Z, Hu L, Zhang J, Yang W, Liu X, Jia D, Yao Z, Chang L, Pan G, Zhong H, *et al*: PCSK9 (Proprotein Convertase Subtilisin/Kexin 9) enhances platelet activation, thrombosis, and myocardial infarct expansion by binding to platelet CD36. Circulation 143: 45-61, 2021.
67. Pignatelli P, Carnevale R, Di Santo S, Bartimoccia S, Sanguigni V, Lenti L, Finocchi A, Mendolicchio L, Soresina AR, Plebani A and Violi F: Inherited human gp91phox deficiency is associated with impaired isoprostane formation and platelet dysfunction. Arterioscler Thromb Vasc Biol 31: 423-434, 2011.
68. Pastori D, Nocella C, Farcomeni A, Bartimoccia S, Santulli M, Vasaturo F, Carnevale R, Menichelli D, Violi F and Pignatelli P; ATHERO-AF Study Group: Relationship of PCSK9 and urinary thromboxane excretion to cardiovascular events in patients with atrial fibrillation. J Am Coll Cardiol 70: 1455-1462, 2017.
69. Schlüter KD, Wolf A, Weber M, Schreckenberg R and Schulz R: Oxidized low-density lipoprotein (oxLDL) affects load-free cell shortening of cardiomyocytes in a proprotein convertase Subtilisin/Kexin 9 (PCSK9)-dependent way. Basic Res Cardiol 112: 63, 2017.
70. Gurbel PA, Navarese EP and Tantry US: Exploration of PCSK9 as a cardiovascular risk factor: Is there a link to the platelet? J Am Coll Cardiol 70: 1463-1466, 2017.
71. Kong N, Xu Q, Cui W, Feng X and Gao H: PCSK9 inhibitor inclisiran for treating atherosclerosis via regulation of endothelial cell pyroptosis. Ann Transl Med 10: 1205, 2022.
72. Wang H and Bai X: Mechanisms of bone remodeling disorder in hemophilia. Semin Thromb Hemost 47: 43-52, 2021.
73. Bovenschen N, Mertens K, Hu L, Havekes LM and van Vlijmen BJ: LDL receptor cooperates with LDL receptor-related protein in regulating plasma levels of coagulation factor VIII in vivo. Blood 106: 906-912, 2005.
74. Paciullo F, Petito E, Falcinelli E, Gresele P and Momi S: Pleiotropic effects of PCSK9-inhibition on hemostasis: Anti-PCSK9 reduce FVIII levels by enhancing LRP1 expression. Thromb Res 213: 170-172, 2022.
75. Strickland DK, Au DT, Cunfer P and Muratoglu SC: Low-density lipoprotein receptor-related protein-1: Role in the regulation of vascular integrity. Arterioscler Thromb Vasc Biol 34: 487-498, 2014.
76. Scalise V, Sanguinetti C, Neri T, Cianchetti S, Lai M, Carnicelli V, Celi A and Pedrinelli R: PCSK9 induces tissue factor expression by activation of TLR4/NFkB signaling. Int J Mol Sci 22: 12640, 2021.
77. Peng J, Liu MM, Liu HH, Guo YL, Wu NQ, Dong Q, Qian J, Dou KF, Zhu CG and Li JJ: Association of circulating proprotein convertase Subtilisin/Kexin type 9 concentration, pro-thrombin time and cardiovascular outcomes: A prospective cohort study. Thromb J 19: 90, 2021.

78. Levine JA, Oleaga C, Eren M, Amaral AP, Shang M, Lux E, Khan SS, Shah SJ, Omura Y, Pamir N, *et al*: Role of PAI-1 in hepatic steatosis and dyslipidemia. Sci Rep 11: 430, 2021.
79. Cronjé HT, Nienaber-Rousseau C, Zandberg L, Chikowore T, de Lange Z, van Zyl T and Pieters M: Candidate gene analysis of the fibrinogen phenotype reveals the importance of polygenic co-regulation. Matrix Biol 60-61: 16-26, 2017.
80. Zhang Y, Zhu CG, Xu RX, Li S, Guo YL, Sun J and Li JJ: Relation of circulating PCSK9 concentration to fibrinogen in patients with stable coronary artery disease. J Clin Lipidol 8: 494-500, 2014.
81. Basiak M, Hachula M, Kosowski M and Okopien B: Effect of PCSK9 inhibitors on hemostasis in patients with isolated hypercholesterolemia. J Clin Med 11: 2542, 2022.
82. Schol-Gelok S, Galema-Boers JAMH, van Gelder T, Kruip MJHA, Roeters van Lennep JE and Versmissen J: No effect of PCSK9 inhibitors on D-dimer and fibrinogen levels in patients with familial hypercholesterolemia. Biomed Pharmacother 108: 1412-1414, 2018.
83. Ahn JH, Tantry US, Kang MG, Park HW, Koh JS, Bae JS, Cho SY, Kim KH, Jang JY, Park JR, *et al*: Residual inflammatory risk and its association with events in east asian patients after coronary intervention. JACC Asia 2: 323-337, 2022.
84. Ridker PM, Everett BM, Thuren T, MacFadyen JG, Chang WH, Ballantyne C, Fonseca F, Nicolau J, Koenig W, Anker SD, *et al*: Antiinflammatory therapy with canakinumab for atherosclerotic disease. N Engl J Med 377: 1119-1131, 2017.
85. Leung AKK, Xue YC, de Guzman A, Grzelkovski G, Kong HJ, Genga KR, Russell JA, Boyd JH, Francis GA and Walley KR: Modulation of vascular endothelial inflammatory response by proprotein convertase Subtilisin-Kexin type 9. Atherosclerosis 362: 29-37, 2022.
86. Raheem Lateef Al-Awsi G, Hadi Lafta M, Hashim Kzar H, Samieva G, Alsaikhan F, Ahmad I, Mahmood Saleh M, Alamin Altoum A, Aravindhan S, Fakri Mustafa Y, *et al*: PCSK9 pathway-noncoding RNAs crosstalk: Emerging opportunities for novel therapeutic approaches in inflammatory atherosclerosis. Int Immunopharmacol 113: 109318, 2022.
87. Kattoor AJ, Goel A and Mehta JL: LOX-1: Regulation, signaling and its role in atherosclerosis. Antioxidants (Basel) 8: 218, 2019.
88. Theofilis P, Sagris M, Antonopoulos AS, Oikonomou E, Tsioufis C and Tousoulis D: Inflammatory mediators of platelet activation: Focus on atherosclerosis and COVID-19. Int J Mol Sci 22: 11170, 2021.
89. Liu ZY, Sun MX, Hua MQ, Zhang HX, Mu GY, Zhou S, Wang Z, Xiang Q and Cui YM: New perspectives on the induction and acceleration of immune-associated thrombosis by PF4 and VWF. Front Immunol 14: 1098665, 2023.
90. Yurtseven E, Ural D, Baysal K and Tokgözoğlu L: An update on the role of PCSK9 in atherosclerosis. J Atheroscler Thromb 27: 909-918, 2020.
91. Ding Z, Liu S, Wang X, Theus S, Deng X, Fan Y, Zhou S and Mehta JL: PCSK9 regulates expression of scavenger receptors and ox-LDL uptake in macrophages. Cardiovasc Res 114: 1145-1153, 2018.
92. Ricci C, Ruscica M, Camera M, Rossetti L, Macchi C, Colciago A, Zanotti I, Lupo MG, Adorni MP, Cicero AFG, *et al*: PCSK9 induces a pro-inflammatory response in macrophages. Sci Rep 8: 2267, 2018.
93. Tang ZH, Peng J, Ren Z, Yang J, Li TT, Li TH, Wang Z, Wei DH, Liu LS, Zheng XL and Jiang ZS: New role of PCSK9 in atherosclerotic inflammation promotion involving the TLR4/NF-κB pathway. Atherosclerosis 262: 113-122, 2017.
94. Puteri MU, Azmi NU, Kato M and Saputri FC: PCSK9 promotes cardiovascular diseases: Recent evidence about its association with platelet Activation-Induced myocardial infarction. Life (Basel) 12: 190, 2022.
95. Vergallo R and Crea F: Atherosclerotic plaque healing. N Engl J Med 383: 846-857, 2020.
96. Laugsand LE, Åsvold BO, Vatten LJ, Janszky I, Platou CG, Michelsen AE, Damås JK, Aukrust P and Ueland T: Circulating PCSK9 and risk of myocardial infarction: The HUNT study in Norway. JACC Basic Transl Sci 1: 568-575, 2016.
97. Kimball AS, Obi AT, Diaz JA and Henke PK: The emerging role of NETs in venous thrombosis and immunothrombosis. Front Immunol 7: 236, 2016.
98. Wang H, Wang Q, Wang J, Guo C, Kleiman K, Meng H, Knight JS and Eitzman DT: Proprotein convertase Subtilisin/Kexin type 9 (PCSK9) deficiency is protective against venous thrombosis in mice. Sci Rep 7: 14360, 2017.
99. Heitzer T, Schlinzig T, Krohn K, Meinertz T and Münzel T: Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. Circulation 104: 2673-2678, 2001.
100. Massberg S, Brand K, Grüner S, Page S, Müller E, Müller I, Bergmeier W, Richter T, Lorenz M, Konrad I, *et al*: A critical role of platelet adhesion in the initiation of atherosclerotic lesion formation. J Exp Med 196: 887-896, 2002.
101. Di Minno A, Gentile M, Iannuzzo G, Calcaterra I, Tripaldella M, Porro B, Cavalca V, Di Taranto MD, Tremoli E, Fortunato G, *et al*: Endothelial function improvement in patients with familial hypercholesterolemia receiving PCSK-9 inhibitors on top of maximally tolerated lipid lowering therapy. Thromb Res 194: 229-236, 2020.
102. Metzner T, Leitner DR, Dimsity G, Gunzer F, Opriessnig P, Mellitzer K, Beck A, Sourij H, Stojakovic T, Deutschmann H, *et al*: Short-Term treatment with alirocumab, flow-dependent dilatation of the brachial artery and use of magnetic resonance diffusion tensor imaging to evaluate vascular structure: An exploratory pilot study. Biomedicines 10: 152, 2022.
103. Itzhaki Ben Zadok O, Mager A, Leshem-Lev D, Lev E, Kornowski R and Eisen A: The effect of proprotein convertase Subtilisin Kexin type 9 inhibitors on circulating endothelial progenitor cells in patients with cardiovascular disease. Cardiovasc Drugs Ther 36: 85-92, 2022.
104. Ding Z, Liu S, Wang X, Deng X, Fan Y, Sun C, Wang Y and Mehta JL: Hemodynamic shear stress via ROS modulates PCSK9 expression in human vascular endothelial and smooth muscle cells and along the mouse aorta. Antioxid Redox Signal 22: 760-771, 2015.
105. Chatzizisis YS, Coskun AU, Jonas M, Edelman ER, Feldman CL and Stone PH: Role of endothelial shear stress in the natural history of coronary atherosclerosis and vascular remodeling: Molecular, cellular, and vascular behavior. J Am Coll Cardiol 49: 2379-2393, 2007.
106. Huang HC, Hsu SJ, Chang CC, Chuang CL, Hou MC and Lee FY: Effects of PCSK-9 Inhibition by alirocumab treatments on biliary cirrhotic rats. Int J Mol Sci 23: 7378, 2022.
107. Wu CY, Tang ZH, Jiang L, Li XF, Jiang ZS and Liu LS: PCSK9 siRNA inhibits HUVEC apoptosis induced by ox-LDL via Bcl/Bax-caspase9-caspase3 pathway. Mol Cell Biochem 359: 347-358, 2012.
108. Li J, Liang X, Wang Y, Xu Z and Li G: Investigation of highly expressed PCSK9 in atherosclerotic plaques and ox-LDL-induced En-Dothelial cell apoptosis. Mol Med Rep 16: 1817-1825, 2017.
109. Guo Y, Tang Z, Yan B, Yin H, Tai S, Peng J, Cui Y, Gui Y, Belke D, Zhou S and Zheng XL: PCSK9 (Proprotein Convertase Subtilisin/Kexin Type 9) triggers vascular smooth muscle cell senescence and apoptosis: Implication of its direct role in degenerative vascular disease. Arterioscler Thromb Vasc Biol 42: 67-86, 2022.
110. O'Leary JG, Greenberg CS, Patton HM and Caldwell SH: AGA clinical practice update: Coagulation in cirrhosis. Gastroenterology 157: 34-43.e1, 2019.
111. Subhani M, Sheth A, Ahmed J, Wijayasiri P, Gardezi SA, Enki D, Morling JR, Aithal GP, Ryder SD and Aravinthan AD: Incidence and prevalence of venous thromboembolism in chronic liver disease: A Sys-Tematic review and meta-analysis. Thromb Res 215: 19-29, 2022.
112. Pant A, Kopec AK and Luyendyk JP: Role of the blood coagulation cascade in hepatic fibrosis. Am J Physiol Gastrointest Liver Physiol 315: G171-G176, 2018.
113. Roberts LN, Patel RK and Arya R: Haemostasis and thrombosis in liver disease. Br J Haematol 148: 507-521, 2010.
114. Northup PG, Garcia-Pagan JC, Garcia-Tsao G, Intagliata NM, Superina RA, Roberts LN, Lisman T and Valla DC: Vascular liver disorders, portal vein thrombosis, and procedural bleeding in patients with liver disease: 2020 practice guidance by the American association for the study of liver diseases. Hepatology 73: 366-413, 2021.
115. Leebeek FW and Rijken DC: The fibrinolytic status in liver diseases. Semin Thromb Hemost 41: 474-480, 2015.
116. Feder S, Wiest R, Weiss TS, Aslanidis C, Schacherer D, Krautbauer S, Liebisch G and Buechler C: Proprotein convertase Subtilisin/Kexin type 9 (PCSK9) levels are not associated with severity of liver disease and are inversely related to cholesterol in a cohort of thirty eight patients with liver cirrhosis. Lipids Health Dis 20: 6, 2021.

117. Grewal T and Buechler C: Emerging insights on the diverse roles of proprotein convertase Subtilisin/Kexin type 9 (PCSK9) in chronic liver diseases: Cholesterol metabolism and beyond. Int J Mol Sci 23: 1070, 2022.
118. Schlegel V, Treuner-Kaueroff T, Seehofer D, Berg T, Becker S, Ceglarek U, Thiery J and Kaiser T: Low PCSK9 levels are correlated with mortality in patients with end-stage liver disease. PLoS One 12: e0181540, 2017.
119. Demers A, Samami S, Lauzier B, Des Rosiers C, Ngo Sock ET, Ong H and Mayer G: PCSK9 induces CD36 degradation and affects long-chain fatty acid uptake and tri-glyceride metabolism in adipocytes and in mouse liver. Arterioscler Thromb Vasc Biol 35: 2517-2525, 2015.
120. Canuel M, Sun X, Asselin MC, Paramithiotis E, Prat A and Seidah NG: Proprotein convertase Subtilisin/Kexin type 9 (PCSK9) can mediate degradation of the low-density lipoprotein receptor-related protein 1 (LRP-1). PLoS One 8: e64145, 2013.
121. Zhang C, Shi X, Su Z, Hu C, Mu X, Pan J, Li M, Teng F, Ling T, Zhao T, *et al*: CD36 deficiency ameliorates drug-induced acute liver injury in mice. Mol Med 27: 57, 2021.
122. Rada P, González-Rodríguez Á, García-Monzón C and Valverde ÁM: Understanding lipotoxicity in NAFLD pathogenesis: Is CD36 a key driver? Cell Death Dis 11: 802, 2020.
123. Kheirkhah A, Lamina C, Kollerits B, Schachtl-Riess JF, Schultheiss UT, Forer L, Sekula P, Kotsis F, Eckardt KU and Kronenberg F; GCKD Investigators: PCSK9 and cardiovascular disease in individuals with moderately decreased kidney function. Clin J Am Soc Nephrol 17: 809-818, 2022.
124. Pavlakou P, Liberopoulos E, Dounousi E and Elisaf M: PCSK9 in chronic kidney disease. Int Urol Nephrol 49: 1015-1024, 2017.
125. Elewa U, Fernández-Fernández B, Mahillo-Fernández I, Martin-Cleary C, Sanz AB, Sanchez-Niño MD and Ortiz A: PCSK9 in diabetic kidney disease. Eur J Clin Invest 46: 779-786, 2016.
126. Artunc F: Kidney-derived PCSK9-a new driver of hyperlipidemia in nephrotic syndrome? Kidney Int 98: 1393-1395, 2020.
127. Lutz J, Menke J, Sollinger D, Schinzel H and Thürmel K: Haemostasis in chronic kidney disease. Nephrol Dial Transplant 29: 29-40, 2014.
128. Lupo MG, Bressan A, Donato M, Canzano P, Camera M, Poggio P, Greco MF, Garofalo M, De Martin S, Panighel G, *et al*: PCSK9 promotes arterial medial calcification. Atherosclerosis 346: 86-97, 2022.
129. Yang W, Zou B, Hou Y, Yan W, Chen T and Qu S: Extracellular vesicles in vascular calcification. Clin Chim Acta 499: 118-122, 2019.
130. Jinnouchi H, Sato Y, Sakamoto A, Cornelissen A, Mori M, Kawakami R, Gadhoke NV, Kolodgie FD, Virmani R and Finn AV: Calcium deposition within coronary atherosclerotic lesion: Implications for plaque stability. Atherosclerosis 306: 85-95, 2020.
131. Ahamad S, Mathew S, Khan WA and Mohanan K: Development of small-molecule PCSK9 inhibitors for the treatment of hypercholesterolemia. Drug Discov Today 27: 1332-1349, 2022.
132. de Ferranti SD, de Boer IH, Fonseca V, Fox CS, Golden SH, Lavie CJ, Magge SN, Marx N, McGuire DK, Orchard TJ, *et al*: Type 1 diabetes mellitus and cardiovascular disease: A scientific statement from the American Heart Association and American Diabetes Association. Circulation 130: 1110-1130, 2014.
133. Feingold KR: Dyslipidemia in diabetes. 2020 Aug 10. In: Feingold KR, Anawalt B, Boyce A, Chrousos G, de Herder WW, Dhatariya K, Dungan K, Hershman JM, Hofland J, Kalra S, *et al*: (eds.). Endotext (Internet). South Dartmouth (MA): MDText.com, Inc.; 2000.
134. Morel O, Jesel L, Abbas M and Morel N: Prothrombotic changes in diabetes mellitus. Semin Thromb Hemost 39: 477-488, 2013.
135. Arnett DK, Blumenthal RS, Albert MA, Buroker AB, Goldberger ZD, Hahn EJ, Himmelfarb CD, Khera A, Lloyd-Jones D, McEvoy JW, *et al*: 2019 ACC/AHA guideline on the primary prevention of cardiovascular disease: Executive summary: A report of the American college of Cardiology/American heart association task force on clinical practice guidelines. Circulation 140: e563-e595, 2019.
136. Kaur R, Kaur M and Singh J: Endothelial dysfunction and platelet hyperactivity in type 2 diabetes mellitus: Molecular insights and therapeutic strategies. Cardiovasc Diabetol 17: 121, 2018.
137. Dregan A, Charlton J, Chowienczyk P and Gulliford MC: Chronic inflammatory disorders and risk of type 2 diabetes mellitus, coronary heart disease, and stroke: A population-based cohort study. Circulation 130: 837-844, 2014.
138. Josefs T, Barrett TJ, Brown EJ, Quezada A, Wu X, Voisin M, Amengual J and Fisher EA: Neutrophil extracellular traps promote macrophage inflammation and impair atherosclerosis resolution in diabetic mice. JCI Insight 5: e134796, 2020.
139. Bryk-Wiązania AH and Undas A: Hypofibrinolysis in type 2 diabetes and its clinical implications: From mechanisms to pharmacological modulation. Cardiovasc Diabetol 20: 191, 2021.
140. Wu Y, Shi J, Su Q, Yang Z and Qin L: Correlation between circulating PCSK9 levels and gestational diabetes mellitus in a Chinese population. Front Endocrinol (Lausanne) 13: 826757, 2022.
141. Saitoski K, Ryaboshapkina M, Hamza GM, Jarnuczak AF, Berthault C, Carlotti F, Armanet M, Sengupta K, Underwood CR, Andersson S, *et al*: Proprotein convertase PCSK9 affects expression of key surface proteins in human pancreatic beta cells via intracellular and extracellular regulatory circuits. J Biol Chem 298: 102096, 2022.
142. Da Dalt L, Ruscica M, Bonacina F, Balzarotti G, Dhyani A, Di Cairano E, Baragetti A, Arnaboldi L, De Metrio S, Pellegatta F, *et al*: PCSK9 deficiency reduces insulin secretion and promotes glucose intolerance: The role of the low-density lipoprotein receptor. Eur Heart J 40: 357-368, 2019.
143. Tchéoubi SER, Akpovi CD, Coppée F, Declèves AE, Laurent S, Agbangla C and Burtea C: Molecular and cellular biology of PCSK9: Impact on glucose homeostasis. J Drug Target 30: 948-960, 2022.
144. Goldman A, Raschi E, Cukierman-Yaffe T, Dankner R, Shouval R, Shechter M, Ben-Zvi I, Gerstein HC and Maor E: Hyperglycaemic disorders associated with PCSK9 inhibitors: A real-world, pharmacovigilance study. Eur J Prev Cardiol 29: 1334-1342, 2022.
145. Marouf BH, Iqbal Z, Mohamad JB, Bashir B, Schofield J, Syed A, Kilpatrick ES, Stefanutti C and Soran H: Efficacy and Safety of PCSK9 monoclonal antibodies in patients with diabetes. Clin Ther 44: 331-348, 2022.
146. Furuhashi M, Sakuma I, Morimoto T, Higashiura Y, Sakai A, Matsumoto M, Sakuma M, Shimabukuro M, Nomiyama T, Arasaki O, *et al*: Differential effects of DPP-4 inhibitors, anagliptin and sitagliptin, on PCSK9 levels in patients with type 2 diabetes mellitus who are receiving statin therapy. J Atheroscler Thromb 29: 24-37, 2022.
147. Khorana AA, Francis CW, Culakova E, Kuderer NM and Lyman GH: Thromboembolism is a leading cause of death in cancer patients receiving outpatient chemotherapy. J Thromb Haemost 5: 632-634, 2007.
148. Kim AS, Khorana AA and McCrae KR: Mechanisms and biomarkers of cancer-associated thrombosis. Transl Res 225: 33-53, 2020.
149. Wolach O and Martinod K: Casting a NET on cancer: The multiple roles for neutrophil extracellular traps in cancer. Curr Opin Hematol 29: 53-62, 2022.
150. Leal AC, Mizurini DM, Gomes T, Rochael NC, Saraiva EM, Dias MS, Werneck CC, Sielski MS, Vicente CP and Monteiro RQ: Tumor-derived exosomes induce the formation of neutrophil extracellular traps: Implications for the establishment of cancer-associated thrombosis. Sci Reps 7: 6438, 2017.
151. Li JC, Zou XM, Yang SF, Jin JQ, Zhu L, Li CJ, Yang H, Zhang AG, Zhao TQ and Chen CY: Neutrophil extracellular traps participate in the development of cancer-associated thrombosis in patients with gastric cancer. World J Gastroenterol 28: 3132-3149, 2022.
152. Fernandes CJ, Morinaga LTK, Alves JL Jr, Castro MA, Calderaro D, Jardim CVP and Souza R: Cancer-associated thrombosis: The when, how and why. Eur Respir Rev 28: 180119, 2019.
153. Taxbro K, Hammarskjöld F, Thelin B, Lewin F, Hagman H, Hanberger H and Berg S: Clinical impact of peripherally inserted central catheters vs implanted port catheters in patients with cancer: An open-label, randomised, two-centre trial. Br J Anaesth 122: 734-741, 2019.
154. Falanga A, Russo L, Milesi V and Vignoli A: Mechanisms and risk factors of thrombosis in cancer. Crit Rev Oncol Hematol 118: 79-83, 2017.
155. Cai YJ, Li PH, Wang XA, Xu YM, Yang S, Tang YN, Zhu Z, Yang XY, He JY, Luo H, *et al*: *Epinephelus coioides* PCSK9 affect the infection of SGIV by regulating the innate immune response. Fish Shellfish Immunol 126: 113-121, 2022.

156. Pirro M, Bianconi V, Francisci D, Schiaroli E, Bagaglia F, Sahebkar A and Baldelli F: Hepatitis C virus and proprotein convertase Subtilisin/Kexin type 9: A detrimental interaction to increase viral infectivity and disrupt lipid metabolism. J Cell Mol Med 21: 3150-3161, 2017.
157. Paciullo F, Fallarino F, Bianconi V, Mannarino MR, Sahebkar A and Pirro M: PCSK9 at the crossroad of cholesterol metabolism and immune function during infections. J Cell Physiol 232: 2330-2338, 2017.
158. Yuan Y, Wu W, Sun S, Zhang Y and Chen Z: PCSK9: A potential therapeutic target for sepsis. J Immunol Res 2020: 2687692, 2020.
159. Barale C, Melchionda E, Morotti A and Russo I: PCSK9 biology and its role in atherothrombosis. Int J Mol Sci 22: 5880, 2021.
160. Zou Y, Chen Z, Zhang X, Yu J, Xu H, Cui J, Li Y, Niu Y, Zhou C, Xia J and Wu J: Targeting PCSK9 ameliorates graft vascular disease in mice by inhibiting NLRP3 inflam-masome activation in vascular smooth muscle cells. Front Immunol 13: 894789, 2022.
161. Sammour Y, Dezorzi C, Austin BA, Borkon AM, Everley MP, Fendler TJ, Khumri TM, Lawhorn SL, Nassif ME, Vodnala D, *et al*: PCSK9 inhibitors in heart transplant patients: Safety, efficacy, and angiographic correlates. J Card Fail 27: 812-815, 2021.
162. Jennings DL, Sultan L, Mingov J, Choe J, Latif F, Restaino S, Clerkin K, Habal MV, Colombo PC, Yuzefpulskaya M, *et al*: PCSK9 inhibitors safely and effectively lower LDL after heart transplantation: A systematic review and meta-analysis. Heart Fail Rev 28: 149-156, 2023.
163. Ortona S, Barisione C, Ferrari PF, Palombo D and Pratesi G: PCSK9 and other metabolic targets to counteract ischemia/reperfusion injury in acute myocardial infarction and visceral vascular surgery. J Clin Med 11: 3638, 2022.
164. Huang G, Lu X, Zhou H, Li R, Huang Q, Xiong X, Luo Z and Li W: PCSK9 inhibition protects against myocardial ischemia-reperfusion injury via suppressing autophagy. Microvasc Res 142: 104371, 2022.