Journal of Autoimmunity 104 (2019) 102347

Contents lists available at ScienceDirect

Journal of Autoimmunity

journal homepage: www.elsevier.com/locate/jautimm

Evidence of epigenetic alterations in thrombosis and coagulation: A systematic review

M.D. Patsouras, P.G. Vlachoyiannopoulos*

Department of Pathophysiology, School of Medicine, National and Kapodistrian University of Athens, Greece

A B S T R A C T

Thrombosis in the context of Cardiovascular disease (CVD) affects mainly the blood vessels supplying the heart, brain and peripheries and it is the leading cause of death worldwide. The pathophysiological thrombotic mechanisms are largely unknown. Heritability contributes to a 30% of the incidence of CVD. The remaining variation can be explained by life style factors such as smoking, dietary and exercise habits, environmental exposure to toxins, and drug usage and other co-morbidities.

Epigenetic variation can be acquired or inherited and constitutes an interaction between genes and the environment. Epigenetics have been implicated in atherosclerosis, ischemia/reperfusion damage and the cardiovascular response to hypoxia. Epigenetic regulators of gene expression are mainly the methylation of CpG islands, histone post translational modifications (PTMs) and microRNAs (miRNAs). These epigenetic regulators control gene expression either through activation or silencing. Epigenetic control is mostly dynamic and can potentially be manipulated to prevent or reverse the uncontrolled expression of genes, a trait that renders them putative therapeutic targets.

In the current review, we systematically studied and present available data on epigenetic alterations implicated in thrombosis derived from human studies. Evidence of epigenetic alterations is observed in several thrombotic diseases such as Coronary Artery Disease and Cerebrovascular Disease, Preeclampsia and Antiphospholipid Syndrome. Differential CpG methylation and specific histone PTMs that control transcription of prothrombotic and proinflammatory genes have also been associated with predisposing factors of thrombosis and CVD, such us smoking, air pollution, hypertriglyceridemia, occupational exposure to particulate matter and comorbidities including cancer, Chronic Obstructive Pulmonary Disease and Chronic Kidney Disease. These clinical observations are further supported by *in vitro* experiments and indicate that epigenetic regulation affects the pathophysiology of thrombotic disorders with potential diagnostic or therapeutic utility.

1. Introduction

1.1. Thrombotic disorders

Thrombosis mainly occurs in the blood vessels supplying the heart, brain and peripheries, resulting in clinical entities which are collectively referred to as cardiovascular disease (CVD). Atheromatous plaque formation, which precedes the thrombotic event, usually occurs in multiple sites and despite the plurality of clinical manifestations (myocardial infarction (MI), stroke, thrombosis and cardiac arrhythmia), these diseases share a common pathophysiological mechanism. Cardiovascular diseases are the world's bigger killers, with 85% of these deaths attributed to heart attack and stroke according to the World Health Organization statistics [1]. Stroke is a clinical syndrome characterized by an acute loss of neurological function, with symptoms lasting longer than 24 h, whereas transient ischemic attack (TIA) lasts less than 24 h without permanent neurological deficit [2]. Ischemic stroke and TIA are usually secondary to thrombosis or embolism of the arteries supplying the brain. Intensive research efforts have revealed several genetic components and environmental predisposing factors, such as smoking, that contribute to the thrombotic process, however key aspects of the thrombotic mechanism, including its deregulation in a pathophysiological context, remain to date largely unknown. Heritability estimates suggest that inherited genetic factors account for approximately 30% of the variation in the incidence of most cardiovascular diseases [3]. The remaining variation is believed to be explained by life style factors such as dietary and exercise habits, environmental exposure to toxins, and drug usage.

Epigenetic variation can be acquired or inherited and constitutes an interaction between genes and the environment. Epigenetic alterations were first described approximately 80 years ago, but mechanistic studies only recently have shed light on the field. The role of epigenetics in determining a range of processes that are believed to be critical in the development and outcome of CVD and thrombosis is being increasingly elaborated [4]. Epigenetic factors have been implicated in influencing atherosclerosis, angiogenesis, ischemia/reperfusion damage, cardiovascular response to hypoxia and fluid shear stress. The reversibility of epigenetic alterations renders them valuable therapeutic targets in the era of precision medicine.

* Corresponding author.
E-mail address: pvlah@med.uoa.gr (P.G. Vlachoyiannopoulos).

https://doi.org/10.1016/j.jaut.2019.102347
Received 29 September 2019; Accepted 3 October 2019
Available online 11 October 2019
0896-8411/

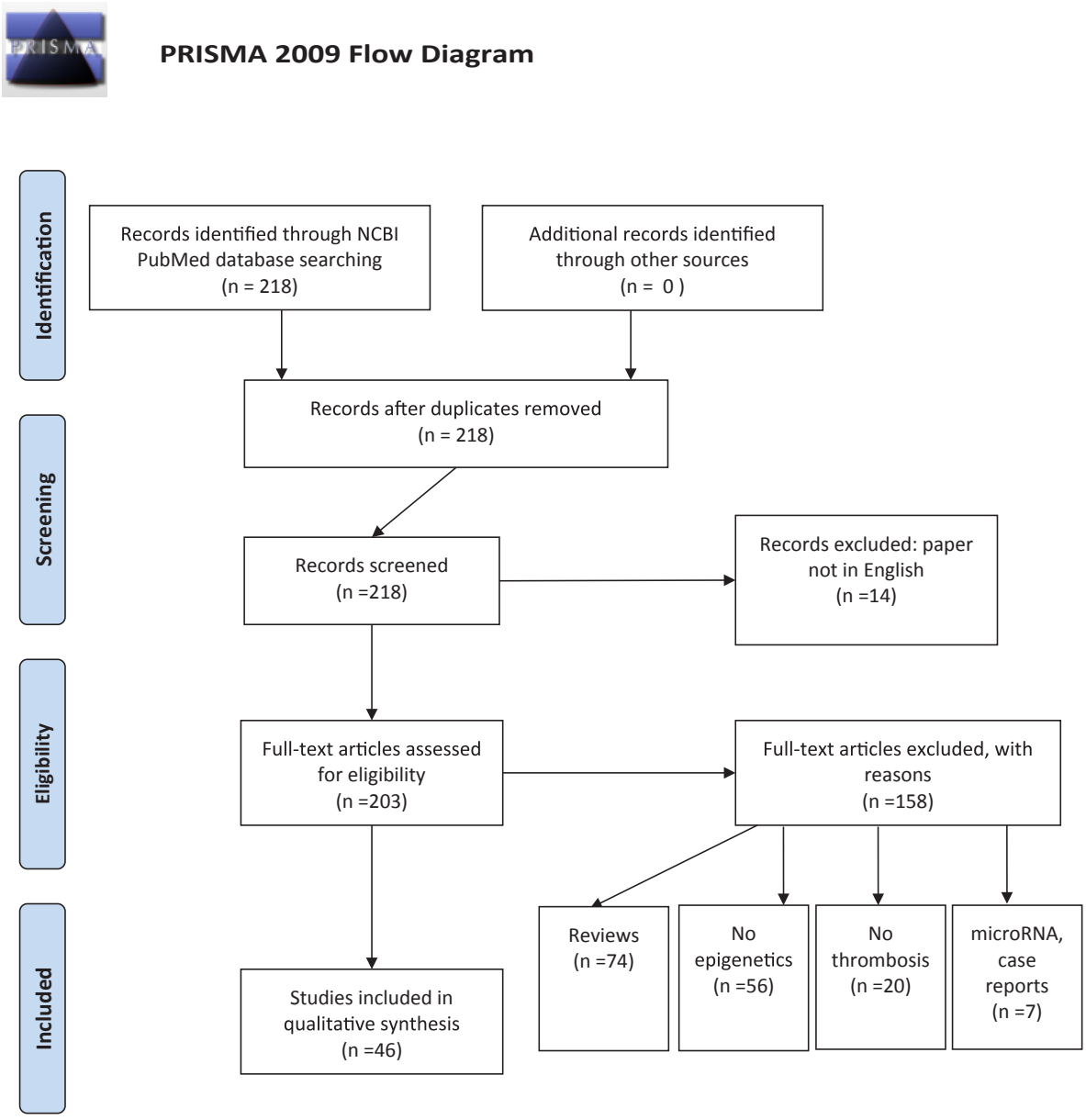

Fig. 1. The flow diagram depicts the flow of information through the different phases of the systematic review.

1.2. Epigenetic regulation of gene expression

Epigenetic regulators of gene expression are mainly the following three: (a) methylation of CpG islands, (b) histone post translational modifications (PTMs) and (c) microRNAs (miRNAs). These epigenetic regulators control gene expression either through activation or silencing. Epigenetic control is mostly dynamic and can potentially be manipulated to prevent or reverse the uncontrolled expression of genes [5].

1.2.1. DNA methylation

The most studied aspect of epigenetics is methylation of DNA at CpG islands. Gene promoter methylation is generally considered to lead to gene silencing. DNA methylation is the process where a methyl group is added to the fifth position of the six-atom ring of cytosine (5-methylcytosine). A group of enzymes known as DNA methyltransferases (DNMTs) catalyze the addition of the methyl group to the cytosine a process which is dynamic [6]. Methyl groups on DNA can be removed either by methyltransferases or by DNA excision and repair [7,8]. DNMTs are subdivided into two groups either for maintenance of DNA methylation (DNMT1) or de novo methylation (DNMT 3a and 3b). DNA methylation results in more compact DNA structure which prevents the interaction of transcription factors with their binding sites. Moreover, methyl binding proteins (MBPs) that bind methylated DNA and subsequently recruit histone deacetylases (HDACs) can result in gene silencing [9].

1.2.2. Histone post-translational modifications (PTMs)

The effect of histone modifications on gene transcription is more complex due to the different types of PTMs. The principal PTMs are acetylation, methylation and phosphorylation [10]. The effect that these modifications have on gene regulation depends on which residue has been modified and to what extent. Histone modifications provide binding sites for several proteins, affecting chromatin configuration gene expression. This cumulative effect is referred as the 'histone code' [11].

The most extensively studied histone PTM is acetylation of lysine residues. Acetylation alters the charge of the histone from positive to

neutral. This process weakens the interaction between histones causing the chromatin to become more accessible. The acetylation is regulated by histone acetyltransferases (HATs) and deacetylation is regulated by HDACs [12,13]. Histone methylation takes place on lysine or arginine residues without affecting the electrical charge of the histones. Either one, two or three methyl-groups can be added on the Lysine residues whereas either one or two on the arginine residues [14], a process linked to both positive and negative gene regulation [11]. Histone phosphorylation changes the charge of the histone proteins from positive to negative. This mark is mostly associated with transcriptional repression [15]. Finally, several other histone PTMs have been reported to a much lesser extent. For example, histones can be ubiquitinated, sumoylated, ADP-ribosylated and glycosylated on lysine residues, citrullinated on arginine residues [16] and proline residues can undergo isomerisation, butyrylation, formylation, 2-hydroxyisobutyrylation malonylation, glutathionylation, succinylation and glutarylation [17].

In the current review, we systematically studied and present available data derived from human studies on epigenetic alterations in thrombosis and coagulation. The main goal of the manuscript is to summarize current knowledge and reveal potential therapeutic targets in the context of thrombotic disorders.

After systematic literature research we identified research papers regarding three main categories: epigenetic alterations observed in the various forms of CVD, epigenetic alterations induced by predisposing factors of CVD and molecular alterations observed in *in vitro* models.

2. Materials and methods

The MEDLINE database was searched in title and abstract for either thrombosis or coagulation in combination with one of the following terms: epigenetic, epigenetics, methylation, hypomethylation, hydroxymethylation, histone deacetylation and histone acetylation. Results were analyzed and 218 unique entries were revealed. No search for miRNAs was performed. From the included studies several concern miRNAs in the context of either methylation or histone PTMs on the gene coding the miRNAs, but not miRNA as an epigenetic regulator per se. After evaluation of the full-texts, 46 studies were included in this review. From the 172 excluded studies 74 were reviews, 56 were irrelevant to epigenetic mechanisms and 20 irrelevant to thrombosis and the rest were in other language or concerned miRNAs, or other species. The inclusion protocol is shown in the Prisma Flow diagram (Fig. 1).

3. Results

3.1. Evidence of epigenetic regulation in cardiovascular disease and atherosclerosis

In 2019, CVD including acute coronary syndrome (ACS) is the leading cause of death worldwide. Data from several studies point to the direction that epigenetic regulation underlies the pathophysiology of CVD.

Two independent studies examining the genome-wide methylation profile in patients with CVD (ACS, stroke, hypertension, thrombosis and cardiac arrhythmia), incorporating a total of 1080 cases revealed differential DNA methylation between patients and controls. Deregulated CpG sites were located in genes implicated in cardiac function, myocardial development cardiovascular disease, cardiogenesis, response to ischemic injury and other processes [18,19]. In purified T and B cells, analysis revealed vital pathways related to atherogenic signaling, adaptive immune response and coronary thrombosis [19]. Another group in the same disease context revealed an inverse correlation between promoter methylation and plasma levels of coagulation factor7 (FVII), a known biomarker of CAD [20] and critical component for the initiation of the intravascular coagulation. FVII forms a complex with tissue factor (TF, also known as FIII) and initiates the intrinsic coagulation cascade explaining why increased clotting activity, due to higher FVII levels, has been associated with a tendency to thrombosis and increased coronary risk [21].

Finally, in monocytes of patients with chronic kidney disease (CKD) and concomitant CAD, the CD40 promoter is hypomethylated and associates with hyperhomocysteinemia, an established CVD risk factor, and the expression of inflammatory molecules such as Tumor necrosis (TNF), Interleukin 6 (IL-6) and Interferon gamma (IFN-γ) [22]. The CD40 and its ligand (CD40-L) form a prothrombotic and proinflammatory system which enhances platelet activation, aggregation, and platelet-leukocyte conjugation contributing thus to the pathophysiology of atherosclerosis and atherothrombosis [23,24].

3.2. Evidence of epigenetic regulation in the pathogenesis of cerebral infarction

Epigenetic alterations have been shown to be involved in cerebral arterial disease as well. In a cohort of 23 patients with atherosclerotic cerebral infarction (ACI) and 32 healthy individuals, methylation of microRNA 223 promoter was significantly lower in ACI patients compared to healthy individuals, significantly lower in patients with carotid atherosclerosis compared to their controls and inversely correlated to miR-223 levels [25]. miR-223 plays an important role in the development of atherosclerosis and ischemic stroke and is involved in processes such as cholesterol metabolism, endothelial cell function, and thrombosis [26].

Moreover patients with cerebral infarction have been found to have higher Thrombomodulin (TM) methylation levels than controls, with a parallel decrease in TM mRNA levels and increase in total homocysteine plasma levels [27]. Thrombomodulin is a particularly important protein for the equilibrium of coagulation and anti-coagulation [28]. It functions as an endothelial cell-surface thrombin receptor and forms a complex with thrombin, leading to the activation of protein C, a serine protease with major anti-coagulant activity [29] which, in turn, proteolytically degrades coagulation factors Va and VIIIa [30]. TM polymorphisms resulting in decreased protein levels significantly associate with increased CAD and venous thromboembolism risk [31].

3.3. Evidence of epigenetic regulation in the pathogenesis of antiphospholipid syndrome

Antiphospholipid syndrome (APS) is an autoimmune thrombophilia which clinically presents as recurrent thromboembolism and/or pregnancy morbidity. Serological markers are the antiphospholipid antibodies, mainly *anti*-β2 glycoprotein I (*anti*-β2GPI) which activate platelets, monocytes and endothelial cells inducing the expression of tissue factor and proinflammatory cytokines, like interleukins 6 (IL6) and 8 (IL8), a process that eventually leads to thrombus formation.

In our study we found that relative methylation was significantly reduced in the IL8 promoter and significantly increased in the tissue factor first intron in APS patients compared to healthy controls, a phenomenon that associated with arterial thrombotic events [32]. In our *in vitro* model simulating APS, *anti*-β2GPI, β2GPI and CXCL4 treatment of both monocytes and endothelial cells also leads to changes in the DNA methylation status of the above genes, and parallel increase of their expression. This information is particularly important because tissue factor, a cell surface glycoprotein that is normally not exposed to blood stream, is the major initiator of the coagulation system. Upon blood vessel injury it is exposed and activates the coagulation factor FVII to FVIIa. Tissue Factor and FVIIa form a complex which activates both FIX and FX. Consequently, activated FX (FXa), along with its cofactor FVa proteolytically cleaves prothrombin to thrombin, which in turn cleaves fibrinogen to fibrin creating a stable fibrin clot [33,34]. Interleukin-8, on the other hand, is an acute phase inflammatory mediator which results in monocyte and neutrophil recruitment and degranulation in sites of increased chemokine concentration. IL8 has been reported to be increased in both atheromatous plaques and serum

of patients with CAD and acute coronary syndrome [35]. In APS treated endothelial cells we observed the transcriptional upregulation of epigenetic factors, including methyl-CpG-binding protein-2 (MECP2), DNA (cytosine-5-)-methyltransferase 3 beta, (DNMT3B), Ten-eleven translocation methylcytosine dioxygenase 1 (TET1), HDAC9 and AT-rich interactive domain-containing protein 5B (ARID5B). These data support that epigenetic regulation could affect the pathophysiological processes in APS with potential diagnostic or therapeutic value [32].

Moreover, in another study in neutrophils isolated from APS, SLE patients and healthy controls, 42 differentially methylated CpG sites were identified. Gene ontology analysis showed the differentially methylated genes to be mainly involved in pregnancy [36].

3.4. Evidence of epigenetic regulation in preeclampsia

Preeclampsia, a clinical entity characterized by new onset of hypertension and proteinuria in pregnancy, is a major cause of maternal mortality and pregnancy morbidity [37]. Pathogenetically, preeclampsia is associated with abnormal activation and consumption of platelets, coagulation factors and the fibrinolytic system [38]. Two different groups have evaluated differential gene methylation in preeclampsia. In two separate studies Mousa et al. assessing the status of DNA methylation in omental arteries identified several genes with reduced DNA methylation in preeclamptic specimens. Gene ontology mapping revealed that smooth muscle contraction, thrombosis and inflammation pathways were overrepresented in the preeclamptic vessels [39]. Particularly relevant identified genes included the thrombin, FV, FXII, GPV, glycoprotein Ib (platelet), alpha polypeptide (GPIBA), Protein C and TBXAS1 genes. The promoter of thromboxane synthase gene (TBXAS1), which is responsible for the synthesis of thromboxane A2, a potent vasoconstrictor and activator of platelets, was decreased whereas thromboxane synthase expression was 2.5-fold increased in omental arteries of preeclamptic women compared to healthy controls [40]. Coagulation factor (FXII) activation is an alternative way for the initiation of intravascular coagulation. Polyphosphates such as adenosine diphosphate (ADP) activate FXII which in turn activates FXI, FIX and eventually FX [41]. Platelet glycoproteins Ib and V are components of the Ib-V-IX system of surface glycoproteins that constitute the receptor for von Willebrand factor which mediates the adhesion of platelets to injured vascular surfaces, focuses the activity of thrombin to the platelet surface and supplies factor VIII (FVIII) to a growing thrombus [42,43]. Therefore, the GPIb-V-IX complex is a central component in the interaction of platelets with von Willebrand Factor (VWF), thrombin and the coagulation end-product fibrin. Another group in maternal leukocyte DNA derived from 14 healthy and 14 preeclamptic women identified differential methylation in genes involved in blood pressure regulation and endothelial homeostasis among which angiotensinogen *(AGT),* calcitonin-related polypeptide alpha (*CALCA)*, and dimethylarginine dimethylamniohydrolase 1 (*DDAH1) genes* [44]. Angiotensinogen (*AGT) is the precursor of* angiotensin II which [45] although principally a potent vasoconstrictor, it also has prothrombotic properties through stimulation of PAI-1 and PAI-2 and consequent platelet aggregation [45,46]. CALCA and dimethylarginine dimethylaminohydrolase (DDAH1) on the other hand mediate potently vessel relaxation and have been implicated in inflammatory response and coronary artery disease respectively [47,48].

The pathways and genes identified from the above studies are depicted in Table 1.

3.5. Evidence of epigenetic regulation in cancer related thrombosis

Thromboembolic clinical incidents characterize several carcinomas [49]. In the next paragraphs we will describe the epigenetic regulation of genes involved in thrombus formation and coagulation in malignancy.

The implication of clear cell carcinoma (CCC) (a histologically distinct carcinoma marked by cytoplasmic clearing, due to accumulation of intracellular glycogen), in thrombotic manifestations through epigenetic regulation has been investigated by two independent groups. Cuff et al. reveal the epigenetic regulation of hepatocyte nuclear factor 1-beta (HNF1B) as a transcription factor of thrombotic genes associated with the blood clotting cascade such as fibrinogen, prothrombin and coagulation factor XIII (FXIII). In their combined gynecologic and renal CCC cohort, they show that both protein levels and hypomethylation of the promoter of the transcription factor were significantly associated with a 2.3-fold increased risk of clinically-significant venous thrombosis [50]. Studying ovarian CCC, Koizume et al. show that the transcriptional activation of the coagulation factor VII (FVII) gene is dependent on histone deacetylation and HDAC4recruitment to the FVII promoter [51].

Another carcinoma type in which thrombotic complications have been demonstrated to be epigenetically regulated is the glioma, a fact attributed to the overexpression of tissue factor and coagulation factor X.

FX and tissue factor promoter methylation levels were found to be lower in glioma specimens and parallel increase in mRNA and protein levels was noted as well, a phenomenon that correlated with overall survival, intratumor microthrombi and venous thromboembolism [52,53]. Interestingly tissue factor overexpression characterized the gliomas that bore the wild type form of an enzyme called isocitrate dehydrogenase 1 (IDH1), whereas the ones bearing a non functional mutant were protected. IDH1 is an enzyme implicated in the citric acid cycle in glucose metabolism and mutations result in a loss of enzymatic function and the consequent abnormal production of 2-hydroxyglutarate (2-HG) [54], which inhibits the enzymatic activity of many dioxygenases, including histone and DNA demethylases, causing widespread changes in histone and DNA methylation [55]. This might explain why in mutant IDH1 gliomas the tissue factor promoter was hypermethylated with a parallel decrease in tissue factor mRNA and protein levels [53], a phenomenon abrogated by the administration of a DNA demethylating agent [56].

Reversely, there is evidence that glioma dormancy is influenced by tissue factor. Tissue factor deficiency leads glioma cells to dormancy but not death, whereas tissue factor expression induces tumor growth. Interestingly, tissue factor expression permanently alters the gene expression and DNA methylation profile of glioma cells that are not longer dormant [57].

Another neoplasm in which patient morbidity and mortality are typically influenced by thrombotic complications is primary myelofibrosis (PMF). In PMF patients the methylation status of CD18 was higher compared to controls, associated with thrombotic complications and proven to be an independent prognostic factor of thrombosis [58]. This piece of information is of value because deregulated expression of the surface leukocyte-specific integrin receptor CD18/CD11b in patient leukocytes facilitates platelet activation, promotes neutrophil recruitment, engagement of platelet glycoprotein GPIbα, endothelial injury and eventually thrombosis [59].

3.6. Evidence of DNA epigenetic alterations in situations predisposing to thrombosis

In the next few paragraphs we will review studies that show that environmental factors or diseases predisposing to thrombosis are characterized by epigenetic alterations of genes involved in coagulation and vascular deregulation.

3.6.1. Environmental factors

Air pollution is a pervasive environmental burden that accounts for approximately 4.2 million deaths every year worldwide, mostly due to cardiovascular disease [60]. Ambient particulate matter (PM) has been associated with increased hospitalization and mortality due to cardiovascular disease in the general population [61]. Moreover, particulate

Table 1
Epigenetic alterations in thrombotic disorders.

Clinical entity	DNA source	Gene affected	Epigenetic alteration	Association with thrombosis
Coronary Artery Disease	whole blood	47 CpGs	differentially methylated	including chemotaxis, coronary thrombosis, and T-cell-mediated cytotoxicity,
	purified T and B cells	several CpGs	differentially methylated	atherogenic signaling and adaptive immune response
	whole blood	211 CpGs	differentially methylated	cardiovascular function, cardiogenesis, responses ischemic injury
	leukocytes	FVII promoter	hypomethylated	plasma levels inversely related to methylation
	monocyte	CD40	hypomethylated	platelet-leukocyte conjugation
Cererebral infarction	leukocytes	miR-223 promoter	hypomethylated	lower in ACI patients than in healthy individuals
		thrombomodulin promoter	hypermethylated	major component of the anticoagulation system
Preeclamsia	omental arteries	TBXAS1 promoter	hypomethylated	platelet activation, potent vasoconstrictor
	omental arteries	Thrombin, FV, FXII, GPV, GPIBA, Protein C	differentially methylated	coagulation and cell adhesion pathways
	leukocytes	POMC, AGT, CALCA, DDAH1	differentially methylated	inflammatory response, vasodilatin, hypertension and endothelial dysfunction
Antiphospholipid syndrome	whole blood	IL8 promoter	hypomethylated	chemotaxis, induces leukocyte adhesion on endothelial cells
	whole blood	Tissue Factor first intron	hypermethylated	initiation of the coagulation cascade
	neutrophils	42 CpGs	hypomethylated	pregnancy and immune response pathways

component of PM has been associated with increased coagulation and risk of venous thrombosis [62].

Occupational exposure to metals leads to differential methylation of several genes related to thrombosis and cardiovascular disease. Work-related zinc and iron PM exposure negatively associated with leukocyte DNA methylation of NOS3 (nitric-oxide-synthase-3), EDN1 (endothelin-1), plasma H3K4me3 and H3K9ac levels, and endogenous thrombin potential (a parameter that reflects the generation of thrombin quantitatively and assesses the coagulability) indicating that NOS3 and EDN1 hypomethylation mediate the effect on coagulation [63,64]. The process of welding is strongly associated with lower whole blood DNA methylation in the proteinase-activated receptor 4 (PAR4) gene which encodes a thrombin receptor [65]. Accordingly, lead exposure significantly inversely correlates with blood DNA methylation in the promoter of glycoprotein IV also known as CD36 [66]. Finally, in a cohort of 704 elderly men, environmental and psychological factors such as air pollution, ambient temperature, relative humidity, life satisfaction and hostility associated with hypomethylation of the promoters of tissue factor, Toll-like receptor 2 (TLR-2), and intercellular adhesion molecule 1 (ICAM-1), iNOS and IFN-γ associated with the presence of all the above parameters, implying a pathogenetic relation of the higher incidence of cardiovascular disease with the exposure to these factors [67–71].

These findings are of particular relevance because all these molecules are part of a network that induces and enhances thrombus formation. PAR4 along with PAR1 are G protein-coupled receptors for thrombin generated by coagulation system, which activates platelets most potently than any other ligand and promotes platelet aggregation and thrombus formation [72]. CD36 is a membrane glycoprotein, present on various monocytes, macrophages, endothelial cells and platelets. Macrophage CD36 participates in the uptake of oxidized LDL (oxLDL), foam cell and atherosclerotic lesion formation [73], while platelet CD36 is necessary for thrombus formation after atherosclerotic plaque rupture [74,75]. ICAM-1 is expressed at sites of endothelial cell activation and permits the stable adhesion and transmigration of circulating leukocytes through its interaction with leukocyte b2 integrins CD11a/CD18 and CD11b/CD18 [76] facilitating thrombus formation in endotoxin induced venous thrombosis [77]. Endothelin-1, a potent vasoconstrictor and pro-inflammatory mediator produced in response to hypoxia, is elevated in patients with CAD [78], co-localizes with atherosclerotic plaque and has been shown to increase coronary inflammation and aggravate myocardial ischemia [79,80]. eNOS, in contrast, is the enzyme that generates Nitric Oxide (NO) in endothelial cells [81], which induces vasodilation and inhibits leukocyte recruitment [82], platelet activation and adhesion to the vessel wall and the already formed thrombus [83]. Finally,TLR2, a pathogen recognition receptor, and the cytokine IFNγ are critical components of innate immunity, which have been shown to render platelets hyperreactive and prothrombotic [84] and to induce the expression of tissue factor and other adhesive molecules and be present in atherosclerotic lesions, respectively [85–87].

3.6.2. Smoking and COPD

Regular smoking is associated with a wide variety of syndromes such as Chronic Obstructive Pulmonary Disease (COPD), cancer, obesity, type 2 diabetes, peripheral arterial disease and coronary artery disease. COPD is the clinical entity characterized by irreversible lung damage due to chronic smoking and bears increased risk for peripheral arterial disease and coronary artery disease [88]. Ongoing research indicates that alterations in the methylation status of DNA in peripheral blood mononuclear cells accompany heavy regular smoking and COPD.

Several groups have performed whole genome methylation analysis in extensive cohorts in DNA isolated from peripheral blood mononuclear cells derived from current smokers and controls. Genes involved in the pathogenesis of cardiovascular disease, inflammation, coagulation and immune response have been identified in all these studies. Thrombin signaling enrichment partially explains the susceptibility to cardiovascular disease that characterizes heavy smokers. One of the most prominent and top ranked differentially methylated genes was the thrombin receptor PAR4 followed by PAR1, von Willebrand Factor and Glycoprotein 5 genes [89–92]. PAR4 DNA methylation was linked to interleukin-18 (IL18) serum levels implying a possible causal relationship [90]. IL18 is a potent proinflammatory cytokine, that has been shown to be an independent predictor of cardiovascular events and to play a role in the formation of atherosclerotic plaque [93,94]. Genome wide methylation analysis in 1454 COPD patients and/or concurrent systemic steroid users yielded similar results. PAR4, PAR3, FXII and CD11b genes were revealed to be differentially methylated [95,96].

The above molecules are cardinal to the pathophysiology of thrombotic disorders. As mentioned above, PAR 1 and 4 when cleaved by thrombin, result in secretion of platelet granule contents including ADP, production of thromboxane A2 (TXA2), and activation of the platelet fibrinogen receptor integrin αIIbβ3 (GPIIb/IIIa) [97–99].

Table 2
Epigenetic alterations in cancer related thrombosis and prothrombotic disorders.

Predisposive setting	thrombosis related gene affected	Epigenetic alteration	Association with thrombosis
Steel workers	EDN 1, NOS 3	promoter hypomethylation	Endothelium deregulation
Welders	PAR4	promoter hypomethylation	Thrombin receptor, platelet activation
Lead	CD36	promoter hypomethylation	Platelet aggregation, atherosclerosis
Air pollution	Tissue Factor,TLR2, ICAM1,IFN γ	promoter hypomethylation	Platelet activation, coagulation
Smoking	PAR4, PAR1, von Willebrand factor, GPV	differential methylation	Coagulation pathway, platelet agreggation and activation
COPD	PAR4, PAR3, FXII and CD11b	differential methylation	Coagulation pathway, platelet agreggation and activation
Diabetes	H4 acetylated	increased levels in monocytes	Possible protective role
Hypertriglyceridemia	PAI-1 promoter	promoter methylation	Fibrinolysis, clot breakdown
Rheumatoid Arthritis	vW, Protein C Inhibitor,	promoter hypomethylation	Platelet adhesion, coagulation
Kawasaki disease	P-selectin, ICAM1, ITGA2B and MAPK14	differential methylation	Platelet activation and adhesion
Psychological state	Tissue factor, ICAM1, TLR2	promoter hypomethylation	Platelet activation, coagulation, fibrinolysis
Temperature and Humidity	Tissue factor, ICAM 1,INOS,TLR2,IFN γ	promoter hypomethylation	Platelet activation, coagulation, fibrinolysis
Chronic Kidney Disease	P-selectin, PAI1, Urokinase, fibrinogen	decrease after BET inhibition	Platelet activation, coagulation, fibrinolysis
Ovarian clear cell carcinoma	HNF1B	promoter hypomethylation	Induces fibrinogen, thrombin and FVIII expression
	FVII	HDAC4 deacetylation	Part of the coagulation cascade
Glioma	FX, Tissue Factor	promoter hypomethylation	Part of the coagulation cascade
Primary Myelofibrosis	CD18	hypermethylated	Cell adhesion and immune response

Activation of PAR1 in the vascular endothelium also leads to increased surface expression of the adhesion molecules intercellular adhesion molecule-1, vascular cell adhesion molecule-1, P-selectin, and E-selectin and gene transcription of cytokines and chemokines such as interleukin-8 [100]. PAR3 is a membrane receptor that facilitates thrombin activation of PAR4 [101].

3.6.3. Diabetes and hypertriglyceridemia

Diabetes and hypertriglyceridemia are known risk factors for CAD and PAD and there is evidence that epigenetics regulate their thrombotic complications. Monocytes from complication free Type 1 diabetic patients have been found to have significantly higher levels of acetylated histone 4 protein compared to healthy controls and patients with diabetic complications, suggesting a potential protective mechanism [102]. Hypertriglyceridemia impairs proper fibrinolytic function in CAD and other atherothrombotic disorders. Plasminogen activator inhibitor-1 (PAI-1), a fibrinolysis inhibitor, is increased by triglyceride-rich lipoproteins in a process dependent on DNA methylation in the PAI-1 promoter [103]. PAI-1 main physiological function is the inhibition of the serine proteases urokinase plasminogen activator (uPA) and tissue plasminogen activator (tPA), enzymes responsible for the cleavage of plasminogen to form plasmin which in turn degrades the fibrin structure of intravascular thrombi [104,105]. PAI-1 deficiencies cause accelerated fibrinolysis and bleeding, whereas elevated PAI-1 plasma levels are associated with vascular thrombosis [106,107].

3.6.4. Systemic autoimmune diseases

Increased risk for CAD in systemic autoimmune diseases has been well established [108]. DNA isolated from patients suffering from rheumatoid arthritis and Kawasaki disease bears a distinctive methyl mark, characteristically different from control samples [109,110]. Among the pathways overrepresented are those of complement, coagulation, platelet activation and inflammation. Particularly relevant to the thrombotic processes are the von Willebrand Factor, Protein C Inhibitor (PCI), P-selectin (SELP), ICAM1, ITGA2B and MAPK14 genes [109,110].

All these molecules participate in cell adhesion and thrombus formation. ITGA2B encodes integrin alpha-IIb which is a component of the GPIIb/IIIa complex on platelets. P-selectin is normally stored in the platelet a granules and endothelial Weibel-Palade bodies [111], and upon platelet and endothelial stimulation rapidly translocates to the cell surface, playing a key role in thrombus formation [112]. P-selectin is critical for thrombus formation since it mediates platelet and leukocyte adhesion in areas of vascular injury and inflammation, an action exerted through its ligand, P-selectin glycoprotein ligand-1 (PSGL-1) [113]. Its soluble form levels are higher in patients with atherosclerosis and thrombosis and associate with future incidence of cerebral and myocardial infarction [114,115]. PCI, on the other hand inhibits not only activated Protein C but also the thrombin-thrombomodulin complex [116]. Finally, MAPK14, also known as p38a, induces inflammatory cytokine expression, regulates platelet activation [117] and plays key roles in atherosclerosis, infarct size, cardiac function and inflammatory response [118].

3.6.5. Chronic kidney disease

Chronic kidney disease (CKD) is characterized by a progressive reduction in glomerular filtration and an increased risk of cardiovascular disease [119].

Apabetalone is an epigenetic modulator which disrupts the interaction between BET proteins and acetylated histones with both *in vitro* and *in vivo* anti-inflammatory and antiatherosclerotic features [120]. In a cohort of patients with CKD stages 4 and 5, the administration of a single dose of apabetaloneresulted in differentially expression of proteins related to inflammation, endothelial dysfunction and coagulation. Among them particularly relevant to thrombosis were IL-6, PAI-1, P-selectin, tPA and uPA, a finding that suggests that cardiovascular complications in CKD are susceptible to epigenetic alteration [121]. The pathways and genes identified from studies of cancer related thrombosis and diseases with increased thrombotic risk are depicted in Table 2.

3.7. Evidence of epigenetic regulation of prothrombotic genes in vitro models

The following studies focus on the epigenetic regulation of gene expression in several cell types based on *in vitro* experiments. Although there is no proof of such mechanisms in actual human diseases these disease models are well established and accepted.

3.7.1. Endothelial cells

Endothelial cell deregulation plays a critical role in thrombotic disorders. For this reason, several groups have studied epigenetic alterations in endothelial cells.

Hypercholesterolemia with a predominant increase of low-density lipoprotein (LDL) cholesterol is a major modifiable risk factor for atherosclerosis and coronary artery disease. In two different studies, LDL treatment of endothelial cells has been proven to induce a prothrombotic phenotype through epigenetic modifications.

LDL treatment affects the expression of the transcription factors KLF2 (Kruppel-like Factor 2) and p66shc through both DNA and histone modification [122,123]. KLF2 levels, which has been proposed to play a protective role against atherothrombosis [124], along with its target genes TM, eNOS and PAI-1 are downregulated by LDL. LDL treatment

stimulates the expression and enzymatic activity of DNMT1, with a parallel binding of methyl CpG binding protein 2 (MECP2) and EZH2 (histone methyltransferase enhancer of zeste homolog 2) on the Krüppel-like Factor 2 (KLF2) promoter, a phenomenon abrogated by DNMT inhibition and EZH2 knockdown [122]. p66shc on the other hand is a transcription factor that mediates hypercholesterolemia-induced endothelial dysfunction and atheromatous plaque formation inducing the expression of ICAM1 and downregulation of TM. LDL stimulates p66shc expression via DNA hypomethylation with concomitant acetylation of histone 3 in its promoter in endothelial cells, an effect negated by DNMT inhibition [123].

Moreover, transcriptional activation of thrombomodulin, human endothelial cell protein C receptor (EPCR) and tissue type plasminogen activator have been proven to be affected by DNA methylation and histone modifications. Retinoic acid treatment of endothelial cells induces the accumulation of trimethylated histone H3K4, a marker for active chromatin, in the TM promoter [125]. EPCR, which is the receptor for protein C that enhances its activation by the thrombin-thrombomodulin (TM) complex [126], contains multiple Specificity protein 1 (Sp1) binding sequences, that are protected from methylation in endothelial cells and collectively contribute to gene expression [127]. Finally, tPA transcription is affected by histone acetylation since HDAC3, HDAC5 and HDAC7 silencing abrogated the transcriptional activation of tPA by valproic acid treatment [128].

3.7.2. Megakaryocytes

Platelets, which are derived from megakaryocytes, are pivotal cells for thrombus formation. ITGA1 gene expression, an integrin encoding gene, is epigenetically repressed in megakaryocytes due to extensive methylation in its promoter and can be de novo induced with the use of a DNA demethylating agent [129]. Although ITGA1 has no proven role in thrombosis, this study indicates that epigenetic regulation affects platelets as well.

The genes identified from the *in vitro* studies are depicted in Table 3.

4. Conclusions

Evidence of epigenetic alterations is observed in several thrombotic diseases such as Coronary Artery Disease, Cerebrovascular Disease, Preeclampsia and Antiphospholipid Syndrome. Differential DNA methylation and histone PTMs that control the transcription of prothrombotic and proinflammatory genes are identified in CVD as well as in correlation with predisposing factors of thrombosis such us smoking, air pollution, hypertriglyceridemia, occupational exposure to particulate matter and comorbidities including cancer, Chronic Obstructive Pulmonary Disease and Chronic Kidney Disease. These clinical observations are further supported by *in vitro* experiments.

At the molecular level, epigenetic modifications regulate major components of coagulation, anticoagulation, fibrinolysis and cellular adhesion during thrombus formation.

All the extrinsic pathway of the coagulation cascade, which comprises of tissue factor exposure on endothelial cells and the serial activation of coagulation factors FVII, FV and FX, thrombin and finally fibrinogen is under epigenetic control. In the intrinsic pathway, the initiator FXII and intermediate factor FVIII are affected as well.

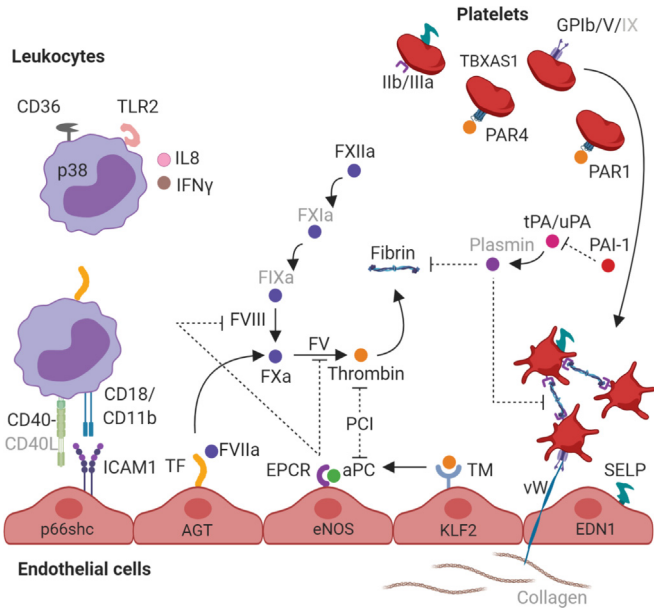

Fig. 2. Schematic representation of thrombotic and coagulation pathways that undergo epigenetic regulation. The use of black font color signifies proven epigenetic modification of the depicted molecules whereas light gray font signifies the lack of evidence of epigenetic regulation. Due to their significance though, these mediators are depicted in the figure. All abbreviations are explained in the main text. The figure was created with BioRender.com.

Gene expression and protein levels of almost the entire anticoagulation system, including the initial thrombin-thrombomodulin complex, the enhancer EPCR, the end product Protein C and its inhibitor PCI, and components of the fibrinolysis pathway, among which are tissue plasminogen activator and its inhibitor PAI1, are affected by epigenetic modifications.

Besides the coagulation process that characterizes the initial step of thrombus formation, several cell surface molecules that mediate platelet and leukocyte adhesion and migration on endothelial cells such as PAR1, PAR4, P-selectin, GPIb/V/IX, CD18/CD11b, CD36, TLR2, ICAM1 and VWF are characterized by differential methylation in several disease settings as well.

Finally, cytokines such us IL8 and IFNγ, vascular tone regulators among which endothelin 1, angiotensinogen, Nitric Oxide Synthase and TBXAS1 and transcription factors such as KLF2, p38α and p66shc are all susceptible to regulation by DNA methylation in thrombotic diseases and disease models. All the above molecules and their annotated position in thrombotic pathways are depicted in Fig. 2.

The above data support that epigenetic regulation affects the pathophysiology of thrombotic disorders with potential diagnostic or therapeutic utility.

Table 3
Epigenetic alterations in *in vitro* models.

Cell type	Gene affected	Epigenetic alteration	Experimental stimulus	Association with thrombosis
Endothelial cells	KLF2	promoter methylation, histone methylation	LDL	Thrombomodulin downregulation and PAI1 overexpression
	p66shc	promoter hypomethylation, H3 acetylation	LDL	Mediates LDL-induced platelet thrombus formation on endothelial cells
	tPA	acetylation of lysines on histones H3 and H4	Valproic Acid	Enhances fibrinolysis pathway
	EPCR	promoter protected from methylation	No treatment	Activated protein C receptor enhanced anticoagulant activity of APC
	Thrombomodulin	H3K4 surrounding the THBD promoter	all-trans Retinoic	major component of the anticoagulation system
Megacaryocytes	ITGA1	promoter methylated	Demethylating agent	none

Funding

This work was supported by the State Scholarships Foundation of Greece (grant number: 3285, March 23, 2016).

Acknowledgments

We thank Dr. Panagiota Karagianni for comments that greatly improved the manuscript.

References

[1] World Health Organization, Cardiovascular Diseases (CVDs), (2017).
[2] American Stroke Association, Types of Stroke, (2011).
[3] A. Baccarelli, S. Ghosh, Environmental exposures, epigenetics and cardiovascular disease, Curr. Opin. Clin. Nutr. Metab. Care 15 (2012) 323–329.
[4] T.F. Whayne, Epigenetics in the development, modification, and prevention of cardiovascular disease, Mol. Biol. Rep. 42 (2015) 765–776.
[5] C.D. Allis, T. Jenuwein, The molecular hallmarks of epigenetic control, Nat. Rev. Genet. 17 (2016) 487–500.
[6] S. Ramchandani, S.K. Bhattacharya, N. Cervoni, M. Szyf, DNA methylation is a reversible biological signal, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 6107–6112.
[7] A. Bird, DNA methylation patterns and epigenetic memory, Genes Dev. 16 (2002) 6–21.
[8] M. Tahiliani, K.P. Koh, Y. Shen, W.A. Pastor, H. Bandukwala, Y. Brudno, et al., Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1, Science 324 (2009) 930–935.
[9] T. Clouaire, I. Stancheva, Methyl-CpG binding proteins: specialized transcriptional repressors or structural components of chromatin? Cell. Mol. Life Sci. : CMLS 65 (2008) 1509–1522.
[10] G.H. Eom, H. Kook, Posttranslational modifications of histone deacetylases: implications for cardiovascular diseases, Pharmacol. Ther. 143 (2014) 168–180.
[11] T. Jenuwein, C.D. Allis, Translating the histone code, Science 293 (2001) 1074–1080.
[12] D. Pons, F.R. de Vries, P.J. van den Elsen, B.T. Heijmans, P.H. Quax, J.W. Jukema, Epigenetic histone acetylation modifiers in vascular remodelling: new targets for therapy in cardiovascular disease, Eur. Heart J. 30 (2009) 266–277.
[13] A. Clocchiatti, C. Florean, C. Brancolini, Class IIa HDACs: from important roles in differentiation to possible implications in tumourigenesis, J. Cell Mol. Med. 15 (2011) 1833–1846.
[14] P.J. Brown, S. Muller, Open access chemical probes for epigenetic targets, Future Med. Chem. 7 (2015) 1901–1917.
[15] D. Rossetto, N. Avvakumov, J. Cote, Histone phosphorylation: a chromatin modification involved in diverse nuclear events, Epigenetics 7 (2012) 1098–1108.
[16] S.B. Rothbart, B.D. Strahl, Interpreting the language of histone and DNA modifications, Biochim. Biophys. Acta 1839 (2014) 627–643.
[17] J. Castillo, G. Lopez-Rodas, L. Franco, Histone post-translational modifications and nucleosome organisation in transcriptional regulation: some open questions, Adv. Exp. Med. Biol. 966 (2017) 65–92.
[18] M. Rask-Andersen, D. Martinsson, M. Ahsan, S. Enroth, W.E. Ek, U. Gyllensten, et al., Epigenome-wide association study reveals differential DNA methylation in individuals with a history of myocardial infarction, Hum. Mol. Genet. 25 (2016) 4739–4748.
[19] J. Li, X. Zhu, K. Yu, H. Jiang, Y. Zhang, S. Deng, et al., Genome-wide analysis of DNA methylation and acute coronary syndrome, Circ. Res. 120 (2017) 1754–1767.
[20] S. Friso, V. Lotto, S.-W. Choi, D. Girelli, M. Pinotti, P. Guarini, et al., Promoter Methylation in Coagulation F7 Gene Influences Plasma FVII Concentrations and Relates to Coronary Artery Disease, (2012).
[21] R. Junker, J. Heinrich, H. Schulte, J. van de Loo, G. Assmann, Coagulation factor VII and the risk of coronary heart disease in healthy men, Arterioscler. Thromb. Vasc. Biol. 17 (1997) 1539–1544.
[22] J. Yang, P. Fang, D. Yu, L. Zhang, D. Zhang, X. Jiang, et al., Chronic kidney disease induces inflammatory CD40+ monocyte differentiation via homocysteine elevation and DNA hypomethylation, Circ. Res. 119 (2016) 1226–1241.
[23] C.D. Garlichs, S. John, A. Schmeisser, S. Eskafi, C. Stumpf, M. Karl, et al., Upregulation of CD40 and CD40 ligand (CD154) in patients with moderate hypercholesterolemia, Circulation 104 (2001) 2395–2400.
[24] B. Pamukcu, G.Y. Lip, V. Snezhitskiy, E. Shantsila, The CD40-CD40L system in cardiovascular disease, Ann. Med. 43 (2011) 331–340.
[25] Z. Li, F. Yu, X. Zhou, S. Zeng, Q. Zhan, M. Yuan, et al., Promoter hypomethylation of microRNA223 gene is associated with atherosclerotic cerebral infarction, Atherosclerosis 263 (2017) 237–243.
[26] A. Rangrez, M. Kumari, N. Frey, An Emerging Role of microRNA miR-223 in Cardiovascular Pathophysiology, (2013).
[27] Z. Yang, L. Wang, W. Zhang, X. Wang, S. Zhou, Plasma Homocysteine Involved in Methylation and Expression of Thrombomodulin in Cerebral Infarction, (2016).
[28] S. Zhang, Z. Zhang, F. Zhang, C. Wei, Y. Bu, S. Zheng, et al., Association between thrombomodulin polymorphisms and coronary artery disease risk: a meta-analysis, Med. Sci. Monit. : Int. Med. J. Exp. Clin. Res. 20 (2014) 1407–1412.
[29] L.V. Mohan Rao, C.T. Esmon, U.R. Pendurthi, Endothelial cell protein C receptor: a multiliganded and multifunctional receptor, Blood 124 (2014) 1553–1562.
[30] F.A. Martin, R.P. Murphy, P.M. Cummins, Thrombomodulin and the vascular endothelium: insights into functional, regulatory, and therapeutic aspects, Am. J. Physiol. Heart Circ. Physiol. 304 (2013) H1585–H1597.
[31] F.A. Martin, R.P. Murphy, P.M. Cummins, Thrombomodulin and the vascular endothelium: insights into functional, regulatory, and therapeutic aspects, Am. J. Physiol. Heart Circ. Physiol. 304 (2013) H1585–H1597.
[32] M.D. Patsouras, P. Karagianni, P. Kogionou, P.G. Vlachoyiannopoulos, Differential CpG methylation of the promoter of interleukin 8 and the first intron of tissue factor in Antiphospholipid syndrome, J. Autoimmun. 102 (August 2019) 159–166.
[33] N. Mackman, The role of tissue factor and factor VIIa in hemostasis, Anesth. Analg. 108 (2009) 1447–1452.
[34] A.P. Owens 3rd, N. Mackman, Tissue factor and thrombosis: the clot starts here, Thromb. Haemost. 104 (2010) 432–439.
[35] S. Apostolakis, K. Vogiatzi, V. Amanatidou, D.A. Spandidos, Interleukin 8 and cardiovascular disease, Cardiovasc. Res. 84 (2009) 353–360.
[36] E. Weeding, P. Coit, S. Yalavarthi, M.J. Kaplan, J.S. Knight, A.H. Sawalha, Genome-wide DNA methylation analysis in primary antiphospholipid syndrome neutrophils, Clin. Immunol. 196 (2018) 110–116.
[37] H.L. Nathan, P.T. Seed, N.L. Hezelgrave, A. De Greeff, E. Lawley, F. Conti-Ramsden, et al., Maternal and perinatal adverse outcomes in women with pre-eclampsia cared for at facility-level in South Africa: a prospective cohort study, J. Global Health 8 (2018) 020401.
[38] K.G. Perry Jr., J.N. Martin Jr., Abnormal hemostasis and coagulopathy in pre-eclampsia and eclampsia, Clin. Obstet. Gynecol. 35 (1992) 338–350.
[39] A.A. Mousa, K.J. Archer, R. Cappello, G. Estrada-Gutierrez, C.R. Isaacs, J.F. Strauss, et al., DNA Methylation Is Altered in Maternal Blood Vessels of Women with Preeclampsia, (2012).
[40] A.A. Mousa, J.F. Strauss, S.W. Walsh, Reduced Methylation of the Thromboxane Synthase Gene Is Correlated with its Increased Vascular Expression in Preeclampsia, (2012).
[41] S. Palta, R. Saroa, A. Palta, Overview of the coagulation system, Indian J. Anaesth. 58 (2014) 515–523.
[42] E. De Candia, S.W. Hall, S. Rutella, R. Landolfi, R.K. Andrews, R. De Cristofaro, Binding of thrombin to glycoprotein Ib accelerates the hydrolysis of Par-1 on intact platelets, J. Biol. Chem. 276 (2001) 4692–4698.
[43] H.J. Weiss, Sussman II, L.W. Hoyer, Stabilization of factor VIII in plasma by the von Willebrand factor. Studies on posttransfusion and dissociated factor VIII and in patients with von Willebrand's disease, J. Clin. Investig. 60 (1977) 390–404.
[44] W.M. White, Z. Sun, K.S. Borowski, B.C. Brost, N.P. Davies, C.H. Rose, et al., Preeclampsia/Eclampsia candidate genes show altered methylation in maternal leukocytes of preeclamptic women at the time of delivery, Hypertens. Pregnancy 35 (2016) 394–404.
[45] H. Kanaide, T. Ichiki, J. Nishimura, K. Hirano, Cellular mechanism of vasoconstriction induced by angiotensin II: it remains to be determined, Circ. Res. 93 (2003) 1015–1017.
[46] T. Skurk, Y.M. Lee, H. Hauner, Angiotensin II and its metabolites stimulate PAI-1 protein release from human adipocytes in primary culture, Hypertension 37 (2001) 1336–1340.
[47] S. Kokkoris, P. Andrews, D.J. Webb, Role of calcitonin gene-related peptide in cerebral vasospasm, and as a therapeutic approach to subarachnoid hemorrhage, Front. Endocrinol. 3 (2012) 135.
[48] H. Kumagai, M. Sakurai, T. Takita, Y. Maruyama, S. Uno, N. Ikegaya, et al., Association of homocysteine and asymmetric dimethylarginine with atherosclerosis and cardiovascular events in maintenance hemodialysis patients, Am. J. Kidney Dis. : Off. J. Natl. Kidney Found. 48 (2006) 797–805.
[49] G. Elyamany, A.M. Alzahrani, E. Bukhary, Cancer-associated thrombosis: an overview, Clin. Med. Insights Oncol. 8 (2014) 129–137.
[50] J. Cuff, K. Salari, N. Clarke, G.E. Esheba, A.D. Forster, S. Huang, et al., Integrative Bioinformatics Links HNF1B with Clear Cell Carcinoma and Tumor-Associated Thrombosis, (2013).
[51] S. Koizume, S. Ito, E. Miyagi, F. Hirahara, Y. Nakamura, Y. Sakuma, et al., HIF2α-Sp1 Interaction Mediates a Deacetylation-dependent FVII-Gene Activation under Hypoxic Conditions in Ovarian Cancer Cells, (2012).
[52] X. Liu, H. Tang, Z. Wang, C. Huang, Z. Zhang, X. She, et al., F10 Gene Hypomethylation, a Putative Biomarker for Glioma Prognosis, (2012).
[53] D. Unruh, S.R. Schwarze, L. Khoury, C. Thomas, M. Wu, L. Chen, et al., Mutant IDH1 and thrombosis in gliomas, Acta Neuropathol. 132 (2016) 917–930.
[54] Z.J. Reitman, H. Yan, Isocitrate dehydrogenase 1 and 2 mutations in cancer: alterations at a crossroads of cellular metabolism, J. Natl. Cancer Inst. 102 (2010) 932–941.
[55] X. Liu, Z.Q. Ling, Role of isocitrate dehydrogenase 1/2 (IDH 1/2) gene mutations in human tumors, Histol. Histopathol. 30 (2015) 1155–1160.
[56] D. Unruh, S. Mirkov, B. Wray, M. Drumm, J. Lamano, Y.D. Li, et al., Methylation-dependent tissue factor suppression contributes to the reduced malignancy of IDH1-mutant gliomas, Clin. Cancer Res. : Off. J. Am. Assoc. Cancer Res. 25 (2019) 747–759.
[57] N. Magnus, D. Garnier, B. Meehan, S. McGraw, T.H. Lee, M. Caron, et al., Tissue Factor Expression Provokes Escape from Tumor Dormancy and Leads to Genomic Alterations, (2014).
[58] C. Augello, D. Cattaneo, C. Bucelli, A. Terrasi, E. Fermo, I. Martinelli, et al., CD18 promoter methylation is associated with a higher risk of thrombotic complications in primary myelofibrosis, Ann. Hematol. 95 (2016) 1965–1969.
[59] J. Hirahashi, K. Hishikawa, S. Kaname, N. Tsuboi, Y. Wang, D.I. Simon, et al., Mac-1 (CD11b/CD18) links inflammation and thrombosis after glomerular injury, Circulation 120 (2009) 1255–1265.
[60] World Health Organization, Mortality and Burden of Disease from Ambient Air Pollution, (2016).

[61] R.D. Brook, S. Rajagopalan, C.A. Pope 3rd, J.R. Brook, A. Bhatnagar, A.V. Diez-Roux, et al., Particulate matter air pollution and cardiovascular disease: an update to the scientific statement from the American Heart Association, Circulation 121 (2010) 2331–2378.
[62] V. Bollati, B. Marinelli, P. Apostoli, M. Bonzini, F. Nordio, M. Hoxha, et al., Exposure to metal-rich particulate matter modifies the expression of candidate microRNAs in peripheral blood leukocytes, Environ. Health Perspect. 118 (2010) 763–768.
[63] L. Tarantini, M. Bonzini, A. Tripodi, L. Angelici, F. Nordio, L. Cantone, et al., Blood Hypomethylation of Inflammatory Genes Mediates the Effects of Metal-Rich Airborne Pollutants on Blood Coagulation, (2013).
[64] L. Cantone, F. Nordio, L. Hou, P. Apostoli, M. Bonzini, L. Tarantini, et al., Inhalable metal-rich air particles and histone H3K4 dimethylation and H3K9 acetylation in a cross-sectional study of steel workers, Environ. Health Perspect. 119 (2011) 964–969.
[65] M.B. Hossain, H. Li, M. Hedmer, H. Tinnerberg, M. Albin, K. Broberg, Exposure to Welding Fumes Is Associated with Hypomethylation of the F2RL3 Gene: a Cardiovascular Disease Marker, (2015).
[66] K. Engström, F. Rydbeck, M. Kippler, T.K. Wojdacz, S. Arifeen, M. Vahter, et al., Prenatal lead exposure is associated with decreased cord blood DNA methylation of the glycoprotein VI gene involved in platelet activation and thrombus formation, Environ. Epigenet. 1 (2015) dvv007.
[67] M.-A. Bind, J. Lepeule, A. Zanobetti, A. Gasparrini, A. Baccarelli, B.A. Coull, et al., Air Pollution and Gene-specific Methylation in the Normative Aging Study: Association, Effect Modification, and Mediation Analysis, (2014).
[68] M.-A. Bind, A. Baccarelli, A. Zanobetti, L. Tarantini, H. Suh, P. Vokonas, et al., Air Pollution and Markers of Coagulation, Inflammation, and Endothelial Function: Associations and Epigene-Environment Interactions in an Elderly Cohort, (2012).
[69] M.-A.C. Bind, B.A. Coull, A. Peters, A.A. Baccarelli, L. Tarantini, L. Cantone, et al., Beyond the Mean: Quantile Regression to Explore the Association of Air Pollution with Gene-specific Methylation in the Normative Aging Study, (2015).
[70] M.-A. Bind, A. Zanobetti, A. Gasparrini, A. Peters, B. Coull, A. Baccarelli, et al., Effects of Temperature and Relative Humidity on DNA Methylation, (2014).
[71] D. Kim, L.D. Kubzansky, A. Baccarelli, D. Sparrow, A. Spiro, L. Tarantini, et al., Psychological factors and DNA methylation of genes related to immune/inflammatory system markers: the VA Normative Aging Study, BMJ open 6 (2016) e009790.
[72] S.R. Coughlin, Thrombin signalling and protease-activated receptors, Nature 407 (2000) 258–264.
[73] Y.M. Park, CD36, a scavenger receptor implicated in atherosclerosis, Exp. Mol. Med. 46 (2014) e99.
[74] M. Yang, B.C. Cooley, W. Li, Y. Chen, J. Vasquez-Vivar, N.O. Scoggins, et al., Platelet CD36 promotes thrombosis by activating redox sensor ERK5 in hyperlipidemic conditions, Blood 129 (2017) 2917–2927.
[75] M.J. Kuijpers, S. de Witt, R. Nergiz-Unal, R. van Kruchten, S.J. Korporaal, P. Verhamme, et al., Supporting roles of platelet thrombospondin-1 and CD36 in thrombus formation on collagen, Arterioscler. Thromb. Vasc. Biol. 34 (2014) 1187–1192.
[76] K.A. Roebuck, A. Finnegan, Regulation of intercellular adhesion molecule-1 (CD54) gene expression, J. Leukoc. Biol. 66 (1999) 876–888.
[77] A.T. Obi, E. Andraska, Y. Kanthi, C.W. Kessinger, M. Elfline, C. Luke, et al., Endotoxaemia-augmented murine venous thrombosis is dependent on TLR-4 and ICAM-1, and potentiated by neutropenia, Thromb. Haemost. 117 (2017) 339–348.
[78] S.B. Chai, X.M. Li, Y.Z. Pang, Y.F. Qi, C.S. Tang, Increased plasma levels of endothelin-1 and urotensin-II in patients with coronary heart disease, Heart Vessel. 25 (2010) 138–143.
[79] M. Fukuchi, A. Giaid, Expression of endothelin-1 and endothelin-converting enzyme-1 mRNAs and proteins in failing human hearts, J. Cardiovasc. Pharmacol. 31 (Suppl 1) (1998) S421–S423.
[80] D. Abraham, M. Dashwood, Endothelin–role in vascular disease, Rheumatology 47 (Suppl 5) (2008) v23–v24.
[81] J.E. Fish, P.A. Marsden, Endothelial nitric oxide synthase: insight into cell-specific gene regulation in the vascular endothelium, Cell. Mol. Life Sci. : CMLS 63 (2006) 144–162.
[82] U. Forstermann, T. Munzel, Endothelial nitric oxide synthase in vascular disease: from marvel to menace, Circulation 113 (2006) 1708–1714.
[83] J.E. Freedman, J. Loscalzo, Nitric oxide and its relationship to thrombotic disorders, J. Thromb. Haemost. : JTH 1 (2003) 1183–1188.
[84] S. Biswas, A. Zimman, D. Gao, T.V. Byzova, E.A. Podrez, TLR2 plays a key role in platelet hyperreactivity and accelerated thrombosis associated with hyperlipidemia, Circ. Res. 121 (2017) 951–962.
[85] J. Kato, T. Okamoto, H. Motoyama, R. Uchiyama, D. Kirchhofer, N. Van Rooijen, et al., Interferon-gamma-mediated tissue factor expression contributes to T-cell-mediated hepatitis through induction of hypercoagulation in mice, Hepatology 57 (2013) 362–372.
[86] N. Luther, F. Shahneh, M. Brahler, F. Krebs, S. Jackel, S. Subramaniam, et al., Innate effector-memory T-cell activation regulates post-thrombotic vein wall inflammation and thrombus resolution, Circ. Res. 119 (2016) 1286–1295.
[87] A. Tedgui, Z. Mallat, Cytokines in atherosclerosis: pathogenic and regulatory pathways, Physiol. Rev. 86 (2006) 515–581.
[88] L. Huiart, P. Ernst, S. Suissa, Cardiovascular morbidity and mortality in COPD, Chest 128 (2005) 2640–2646.
[89] M.V. Dogan, B. Shields, C. Cutrona, L. Gao, F.X. Gibbons, R. Simons, et al., The Effect of Smoking on DNA Methylation of Peripheral Blood Mononuclear Cells from African American Women, (2014).
[90] M.A. Jhun, J.A. Smith, E.B. Ware, S.L.R. Kardia, T.H. Mosley, S.T. Turner, et al., Modeling the causal role of DNA methylation in the association between cigarette smoking and inflammation in African Americans: a 2-step epigenetic mendelian randomization study, Am. J. Epidemiol. 186 (2017) 1149–1158.
[91] N.S. Shenker, S. Polidoro, K.v. Veldhoven, C. Sacerdote, F. Ricceri, M.A. Birrell, et al., Epigenome-wide Association Study in the European Prospective Investigation into Cancer and Nutrition (EPIC-Turin) Identifies Novel Genetic Loci Associated with Smoking, (2013).
[92] A. Chatziioannou, P. Georgiadis, D.G. Hebels, I. Liampa, I. Valavanis, I.A. Bergdahl, et al., Blood-based omic profiling supports female susceptibility to tobacco smoke-induced cardiovascular diseases, Sci. Rep. 7 (2017) 42870.
[93] S. Blankenberg, G. Luc, P. Ducimetiere, D. Arveiler, J. Ferrieres, P. Amouyel, et al., Interleukin-18 and the risk of coronary heart disease in european men: the prospective epidemiological study of myocardial infarction (PRIME), Circulation 108 (2003) 2453–2459.
[94] Z. Mallat, A. Corbaz, A. Scoazec, S. Besnard, G. Leseche, Y. Chvatchko, et al., Expression of interleukin-18 in human atherosclerotic plaques and relation to plaque instability, Circulation 104 (2001) 1598–1603.
[95] W. Qiu, A. Baccarelli, V.J. Carey, N. Boutaoui, H. Bacherman, B. Klanderman, et al., Variable DNA Methylation Is Associated with Chronic Obstructive Pulmonary Disease and Lung Function, (2012).
[96] E.S. Wan, W. Qiu, A. Baccarelli, V.J. Carey, H. Bacherman, S.I. Rennard, et al., Systemic Steroid Exposure Is Associated with Differential Methylation in Chronic Obstructive Pulmonary Disease, (2012).
[97] A. Abdel-Latif, S.S. Smyth, Preventing platelet thrombosis with a PAR1 pepducin, Circulation 126 (2012) 13–15.
[98] L. Covic, A.L. Gresser, A. Kuliopulos, Biphasic kinetics of activation and signaling for PAR1 and PAR4 thrombin receptors in platelets, Biochemistry 39 (2000) 5458–5467.
[99] S.J. Joo, Mechanisms of platelet activation and integrin alphaIIbeta3, Korean Circ. J. 42 (2012) 295–301.
[100] T. Minami, A. Sugiyama, S.Q. Wu, R. Abid, T. Kodama, W.C. Aird, Thrombin and phenotypic modulation of the endothelium, Arterioscler. Thromb. Vasc. Biol. 24 (2004) 41–53.
[101] M. Nakanishi-Matsui, Y.-W. Zheng, D.J. Sulciner, E.J. Weiss, M.J. Ludeman, S.R. Coughlin, PAR3 is a cofactor for PAR4 activation by thrombin, Nature 404 (2000) 609–613.
[102] S.S.H. Chen, A.J. Jenkins, H. Majewski, Elevated plasma prostaglandins and acetylated histone in monocytes in Type 1 diabetes patients, Diabet. Med. : J. Br. Diabet. Assoc. 26 (2009) 182–186.
[103] P. Eriksson, L. Nilsson, F. Karpe, A. Hamsten, Very-low-density lipoprotein response element in the promoter region of the human plasminogen activator inhibitor-1 gene implicated in the impaired fibrinolysis of hypertriglyceridemia, Arterioscler. Thromb. Vasc. Biol. 18 (1998) 20–26.
[104] D.E. Vaughan, PAI-1 and atherothrombosis, J. Thromb. Haemost. : JTH 3 (2005) 1879–1883.
[105] D.C. Rijken, H.R. Lijnen, New insights into the molecular mechanisms of the fibrinolytic system, J. Thromb. Haemost. : JTH 7 (2009) 4–13.
[106] M. Cesari, M. Pahor, R.A. Incalzi, Plasminogen activator inhibitor-1 (PAI-1): a key factor linking fibrinolysis and age-related subclinical and clinical conditions, Cardiovasc. Ther. 28 (2010) e72–91.
[107] D. Collen, H.R. Lijnen, The tissue-type plasminogen activator story, Arterioscler. Thromb. Vasc. Biol. 29 (2009) 1151–1155.
[108] I. Hollan, P.L. Meroni, J.M. Ahearn, J.W. Cohen Tervaert, S. Curran, C.S. Goodyear, et al., Cardiovascular disease in autoimmune rheumatic diseases, Autoimmun. Rev. 12 (2013) 1004–1015.
[109] J.W. Whitaker, R. Shoemaker, D.L. Boyle, J. Hillman, D. Anderson, W. Wang, et al., An imprinted rheumatoid arthritis methylome signature reflects pathogenic phenotype, Genome Med. 5 (2013) 40.
[110] D. Chang, C. Qian, H. Li, H. Feng, Comprehensive analyses of DNA methylation and gene expression profiles of Kawasaki disease, J. Cell. Biochem. 120 (8) (August 2019) 13001–13011.
[111] R. Bonfanti, B.C. Furie, B. Furie, D.D. Wagner, PADGEM (GMP140) is a component of Weibel-Palade bodies of human endothelial cells, Blood 73 (1989) 1109–1112.
[112] D.D. Wagner, P.C. Burger, Platelets in inflammation and thrombosis, Arterioscler. Thromb. Vasc. Biol. 23 (2003) 2131–2137.
[113] P.S. Frenette, C. Moyna, D.W. Hartwell, J.B. Lowe, R.O. Hynes, D.D. Wagner, Platelet-endothelial interactions in inflamed mesenteric venules, Blood 91 (1998) 1318–1324.
[114] G.S. Hillis, C. Terregino, P. Taggart, A. Killian, N. Zhao, W.C. Dalsey, et al., Elevated soluble P-selectin levels are associated with an increased risk of early adverse events in patients with presumed myocardial ischemia, Am. Heart J. 143 (2002) 235–241.
[115] P.M. Ridker, J.E. Buring, N. Rifai, Soluble P-selectin and the risk of future cardiovascular events, Circulation 103 (2001) 491–495.
[116] A.R. Rezaie, S.T. Cooper, F.C. Church, C.T. Esmon, Protein C inhibitor is a potent inhibitor of the thrombin-thrombomodulin complex, J. Biol. Chem. 270 (1995) 25336–25339.
[117] P. Shi, L. Zhang, M. Zhang, W. Yang, K. Wang, J. Zhang, et al., Platelet-specific p38alpha deficiency improved cardiac function after myocardial infarction in mice, Arterioscler. Thromb. Vasc. Biol. 37 (2017) e185–e196.
[118] J. Ren, S. Zhang, A. Kovacs, Y. Wang, A.J. Muslin, Role of p38alpha MAPK in cardiac apoptosis and remodeling after myocardial infarction, J. Mol. Cell. Cardiol. 38 (2005) 617–623.
[119] R.T. Gansevoort, R. Correa-Rotter, B.R. Hemmelgarn, T.H. Jafar, H.J. Heerspink, J.F. Mann, et al., Chronic kidney disease and cardiovascular risk: epidemiology, mechanisms, and prevention, Lancet 382 (2013) 339–352.

[120] S.J. Nicholls, K.K. Ray, J.O. Johansson, A. Gordon, M. Sweeney, C. Halliday, et al., Selective BET protein inhibition with apabetalone and cardiovascular events: a pooled analysis of trials in patients with coronary artery disease, Am. J. Cardiovasc. Drugs : Drugs, Devices Interv. 18 (2018) 109–115.
[121] S. Wasiak, L.M. Tsujikawa, C. Halliday, S.C. Stotz, D. Gilham, R. Jahagirdar, et al., Benefit of apabetalone on plasma proteins in renal disease, Kidney Int. Rep. 3 (2018) 711–721.
[122] A. Kumar, S. Kumar, A. Vikram, T.A. Hoffman, A. Naqvi, C.M. Lewarchik, et al., Histone and DNA methylation-mediated epigenetic downregulation of endothelial Kruppel-like factor 2 by low-density lipoprotein cholesterol, Arterioscler. Thromb. Vasc. Biol. 33 (2013) 1936–1942.
[123] Y.-R. Kim, C.-S. Kim, A. Naqvi, A. Kumar, S. Kumar, T.A. Hoffman, et al., Epigenetic Upregulation of P66shc Mediates Low-Density Lipoprotein Cholesterol-Induced Endothelial Cell Dysfunction, (2012).
[124] M.A. Gimbrone Jr., G. Garcia-Cardena, Vascular endothelium, hemodynamics, and the pathobiology of atherosclerosis, Cardiovasc. Pathol. : Off. J. Soc. Cardiovasc. Pathol. 22 (2013) 9–15.
[125] Z. Li, B. Chen, X. Weng, L. Yu, M. Song, M. Fang, et al., The histone methyltransferase SETD1A regulates thrombomodulin transcription in vascular endothelial cells, Biochim. Biophys. Acta Gene Regul. Mech. 1861 (2018) 752–761.
[126] D.J. Stearns-Kurosawa, S. Kurosawa, J.S. Mollica, G.L. Ferrell, C.T. Esmon, The endothelial cell protein C receptor augments protein C activation by the thrombin-thrombomodulin complex, Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 10212–10216.
[127] J.B. Rance, G.A. Follows, P.N. Cockerill, C. Bonifer, D.A. Lane, R.E. Simmonds, Regulation of the human endothelial cell protein C receptor gene promoter by multiple Sp1 binding sites, Blood 101 (2003) 4393–4401.
[128] P. Larsson, E. Ulfhammer, M. Magnusson, N. Bergh, S. Lunke, A. El-Osta, et al., Role of Histone Acetylation in the Stimulatory Effect of Valproic Acid on Vascular Endothelial Tissue-type Plasminogen Activator Expression, (2012).
[129] Y. Cheli, S. Kanaji, B. Jacquelin, M. Chang, D.J. Nugent, T.J. Kunicki, Transcriptional and epigenetic regulation of the integrin collagen receptor locus ITGA1-PELO-ITGA2, Biochim. Biophys. Acta 1769 (2007) 546–558.