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Review Article

Interplay between genetics and epigenetics in modulating the risk of venous thromboembolism: A new challenge for personalized therapy

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ABSTRACT

Although venous thromboembolism (VTE) shows a polygenic nature, the crossroad between genome and environment is not fully understood. Genetics explains only a part of VTE hereditability and not defined molecular causes are found in approximately 50% of thrombotic patients. Thus, a major understanding of molecular mechanisms may clarify the missing hereditability. Concerning epigenetics, a particular histone modification (citrullination) plays a key role in increasing the rate of venous occlusive events by inducing neutrophil apoptosis and expulsion of neutrophil extra-cellular traps (NETs), which may be useful biomarkers of active disease. Moreover, an over-expression of miR-320a/b, miR-582, miR-195, miR-424-5p, and miR-532, or a down-regulation of miR495, miR-136-5p and miR-26a may improve the accuracy of VTE diagnosis. No clinical studies have focused on DNA methylation in VTE. Nowadays, no validated epigenetics biomarkers are routinely used for diagnosis and prevention of VTE. In the era of personalized therapy, several clinical trials are investigating the putative role of statins, a class of lipid-lowering epigenetic-based drugs, as additional therapeutic agents in VTE. Furthermore, single nucleotide polymorphisms (SNPs) in *CYP2C9*, *VKORC1*, and *MIR133* genes can help physicians to predict individual warfarin dose requirement. Consequently, a comprehensive understanding of the mechanisms involved in the control of blood clot development is crucial to design novel therapeutic strategies. This review summarizes the current clinical concepts both in genetic and epigenetic VTE framework. Furthermore, we discuss the contribution of the innovative network medicine paradigm into advancing our knowledge about molecular underpinnings needed to support novel VTE diagnostic and therapeutic options.

1. Introduction

Venous thromboembolism (VTE) and cardiovascular (CV) disorders share some common risk factors associated with high mortality rate worldwide [1]. VTE includes deep vein thrombosis (DVT) of the legs and its pulmonary embolism (PE), its major complication [1]. VTE occurs with an annual incidence of about 1–3 subjects per 1000, although the prevalence is increasing with aging and Western lifestyles [1]. Clinical studies highlighted that VTE is a complex and multifactorial disease caused by numerous interactions between genome and environmental exposures [2–4]. Indeed, > 40% of VTE patients present one or more predisposing genetic and/or acquired risk factors that synergistically contribute to the individual "thrombosis potential" [5,6]. However, VTE determinants are highly heterogeneous and, for some of them, both the magnitude and independence are still uncertain [5,6]. The inherited risk factors include a group of abnormalities switching the hemostatic balance *vs* a hypercoagulability state [6]. Family-based approach suggested that > 60% of VTE susceptibility is attributable to genetic factors [7]; however, these ones account only for a small part (about 5%) of the hereditability and about 50% of thrombotic patients has no detectable gene alterations [6,7]. The main acquired risk factors are all forms of prolonged immobilization, major surgery, pregnancy, oral contraceptive use, acquired resistance to protein C (independently from FV Leiden), hormone replacement therapy, obesity, cancer, as well as immune-system diseases [5]. VTE also occurs without clear risk factors (idiopathic form), thus suggesting the necessity of a deeper

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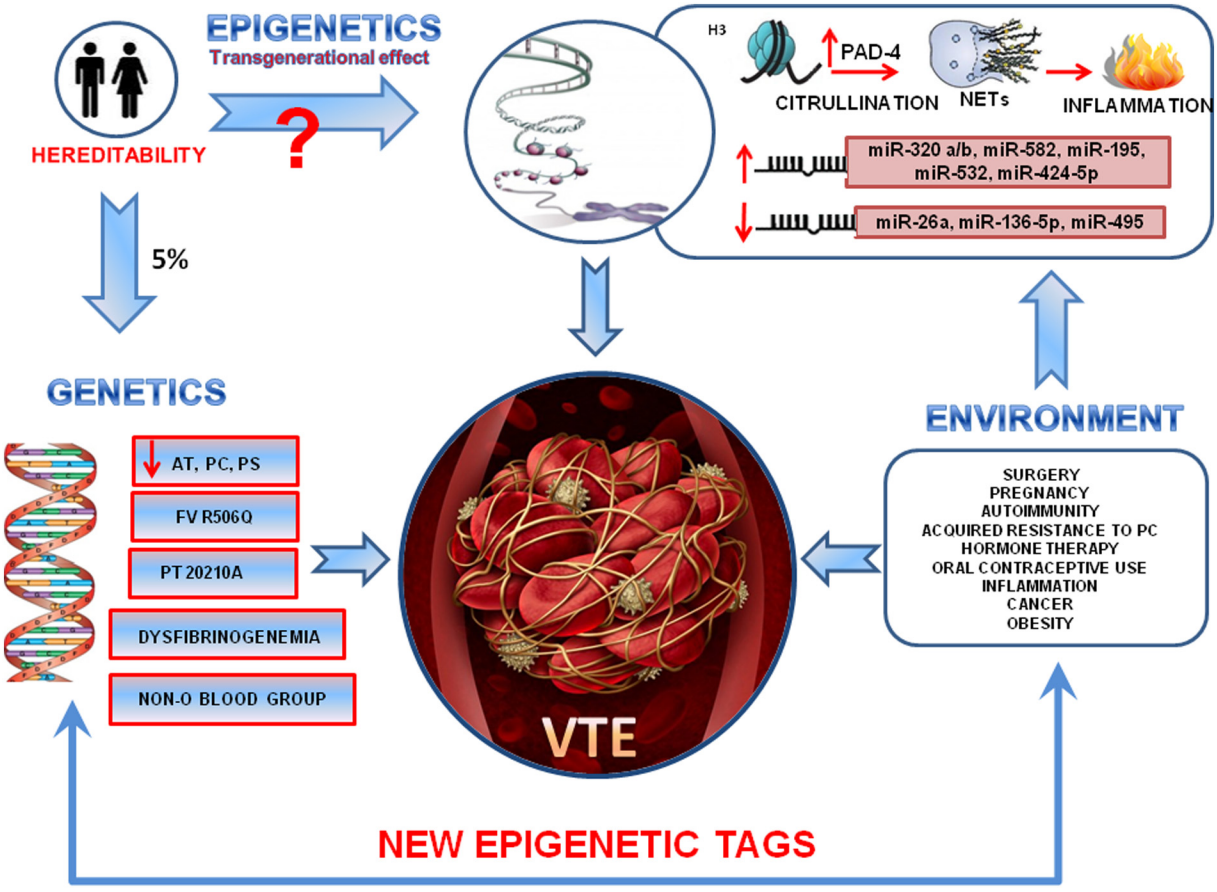

Fig. 1. Crossroads of genetics, epigenetics, and environment in modulating the risk of VTE. Rare mutations causing deficiencies of natural anticoagulants (AT, PC, PC), common SNPs leading to pro-coagulant effect (FV R506Q, PT 2210A), dysfibrinogenemia, as well as non-O blood group are well-established risk factors for thrombotic events. However, all these ones explain only about 5% of expected sensitiveness. Epigenetic-sensitive pathways are stable and reversible changes by which cells respond to environmental stress. Epigenome may contribute to VTE missing hereditability but its magnitude is yet unknown. Neutrophils, by up-regulation of PAD-4-mediated citrullination, undergo a histone remodelling leading to extrusion of NETs. They are fibers of DNA complexed with histones and proteins of granules such as serine proteases, cathepsin G, and myeloperoxidase, which trigger a hypercoagulability state. Besides, an up-regulation of miR-320a/b, miR-582, miR-195, miR-532, and miR-424-5p as well as a down-regulation of miR-495, miR-136-5p and miR-26a are associated with high VTE risk. Acquired thrombophilia is explained by transient environmental risk factors including all forms of immobilization, pregnancy, cancer, obesity, autoimmunity, hormone therapy, and inflammation. All these conditions can modify gene expression by enhancing abovementioned epigenetic tags or triggering novel changes during life, yet unknown. Then, epigenetic mechanisms bridge inherited and acquired risk factors in subjects genetically predisposed to develop VTE and could be useful diagnostic biomarkers as well as innovative target therapies.

understanding about molecular defects altering crucial genes or pathways in the cellular interactome [5,6]. Since epigenetic mechanisms (DNA methylation, histone modifications, and non-coding RNAs) are key players in many CV diseases, they may also explain the "missing heritability" in VTE onset [8–12].

This review aims to update both on genetic and epigenetic mechanistic aspects of VTE onset. Furthermore, we discuss the innovative paradigm of network medicine that ideally overcomes the shortcomings of the current reductionist approach and might shed new lights into VTE clinical setting.

2. Hereditability of VTE

VTE genetic risk factors play the major pathogenic role in younger patients (< 50 years) [7]. The major classes are: 1) rare loss-of-function (LOF) mutations in antithrombin (*SERPINC1*), protein C (*PROC*), and its cofactor protein S (*PROS1*) genes, 2) common gain-of-function (GOF) single nucleotide polymorphisms (SNPs) in factor V Leiden, 3) prothrombin (G20210A) SNP, and 4) and dysfibrinogenemia [7]. In addition, non-O blood group is considered strongly associated with high risk of VTE [6] (Fig. 1). Rare abnormalities (< 1%) are due to hundreds of molecular mechanisms resulting into private mutations that destroy *SERPINC1*, *PROC*, and *PROS1* gene expression [13]. These alterations are associated with an increased VTE risk for heterozygous carriers and show high penetrance. For this reason, they are classified as strong VTE risk factors [6]. In contrast, factor V Leiden, prothrombin 20210A mutation, and non-O blood group are classified as moderate risk factors [6]. In detail, factor V Leiden is caused by a SNP replacing a guanine with an adenine at nucleotide position 1691 of factor V (*FV*) gene. This SNP causes a missense substitution thereby arginine is changed with glutamine at position 506 (FV R506Q) of exon 10 in factor V activated (FVa) [14]. This change alters the major cleavage site used by the activate protein C (APC) to modulate coagulation [14]. As consequence, FVa resists to activated protein C (APC-resistance) and triggers a persistent activation of prothrombin. Factor V Leiden is related to a five-fold and fifty-fold increased risk in heterozygotes and homozygotes carriers, respectively [6]. Although moderate, factor V Leiden is much frequent (about 5%) in Caucasian population and is responsible for venous thrombotic events in a large proportion (about 20–25%) [6]. Prothrombin 20210A is caused by a SNP replacing a guanine with an adenine at nucleotide position 20,210 in 3′ untranslated region (3′UTR) of *FII* gene [15]. This variation alters the binding site of poly-A tail leading to a major expression of *FII*. The risk is increased from two to three-fold in prothrombin 20210A carriers and it is found in about 6%

of VTE patients [6]. Moreover, subjects with non-O group are associated with a two to four-fold increased risk of VTE compared to O-group individuals likely due to higher plasma levels of von Willenbrand factor (vWf) and factor VIII [16]. Besides the aforementioned biomarkers, several SNPs are classified as weak risk factors because they are highly frequent in population but have a small (ranging from 1.0 to 1.5) effect on the risk [6]. One of the most common weak SNP replaces cytidine with tymidine at position 677 (C677T) of 5,10-methyltetrahydrofolate reductase (*MTHFR*) gene [17]. *MTHFR* is involved in homocysteine metabolism and catalyzes the reduction of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the major circulating form of folate and carbon donor for the re-methylation of homocysteine to methionine [17]. This variant causes a LOF mutation thereby an alanine is changed by a valine resulting in a thermolabile enzyme less active at higher temperatures. As consequence, plasma levels of homocysteine are mildly more elevated, thus causing a major risk of vessel wall injury, especially in subjects who are simultaneous deficient in folate [17]. Thus, the interaction between genetics and environmental/nutritional conditions is crucial for homocysteine concentrations both in healthy and vascular disease. Approximately 10–15% of Caucasians is carrier homozygous of MTHFR 677TT genotype that is associated with a 20% higher risk of VTE compared to MTHFR 677CC genotype [18]. Indeed, a mild hyper-homocysteinemia was diagnosed in at least 40% of VTE patients, which were treated with a daily supplementation of low folic acid dose [17].

In the era of next generation sequencing (NGS), the development of potent genome wide association studies (GWAS), whole exosome sequencing (WES), and whole genome sequencing (WGS) platforms may enlarge the window of responsiveness genes, thus improving the performance of genetic VTE screening. Some GWAS confirmed the associations between VTE and SNPs in coagulation factor V (FV), alpha 1–3-*N*-acetylgalactosaminyltransferase and alpha 1–3-galactosyltransferase (*ABO*), coagulation factor XI (*FXI*), fibrinogen gamma chain (*FGG*), and coagulation factor II (*FII*) genes [19–21]. The discovery of SNPs in novel susceptibility loci, such as glycoprotein VI (*GP6*), cytochrome P450 family 4 subfamily V member 2 (*CYP4V2*), vWF, syntaxin binding protein 5 (*STXBP5*), and kininogen 1 (*KNG1*) was successful results of sequencing approach; however, these determinants still require an accurate clinical validation [19,22–24]. The etiology of VTE is not restricted to the coagulation system but, rather, an inflammation plays a crucial role by exacerbating the clinical status of VTE patients [25]. GWAS studies revealed the genetic basis about the involvement of inflammation in VTE [22]. Indeed, some evidence reported that −1082A/G SNP in the interleukin (IL)-10 (*IL-10*), as well as the allele rs169713C of the human immunodeficiency virus type I enhancer binding protein 1 (*HIVEP1*) were associated with higher risk of VTE [22,26]. Biochemical studies also indicated a correlation between VTE and several inflammatory markers, such as C-reactive protein (CRP), IL-6, IL-8, and tumor necrosis factor-alpha (TNF-α) [27]. To date, VTE clinical management is largely based on oral anticoagulants as warfarin, in order to reduce thrombus size and prevent its extension; however, no therapeutic strategy is designed to inhibit inflammation and no related biomarkers are yet established. A major knowledge in this field may offer a potential anti-inflammatory strategy to avoid warfarin bleeding risk. Compared to GWAS, WES and WGS platforms can identify extremely rare (< 1%) or unknown mutations that may explain the high proportion of idiopathic VTE. However, the only WES application provided only partial results [28]. Instead, WGS platforms were limited to coding regions of 186 genes previously associated to alteration of coagulation cascade [28–30].

3. Epigenome: the “missing heritability” of VTE?

Epigenetics is typically defined as the study of heritable changes in gene expression without variations in DNA sequence [31]. The main classes of epigenetic regulators are: DNA and mRNA methylation, histone modifications, as well as noncoding RNA action [31,32]. Epigenetic mechanisms persist during cell division and provide heritability and diversity to the cellular phenotype acting through chromatin modifications with consequent positive or negative modulation of gene expression [31]. In essence, epigenetic changes rearrange chromatin into euchromatin or heterochromatin areas leading to gene activation and gene repression, respectively. Epigenome maintains cellular identity and is specific for the spatial-temporal status, although it responds to environment changes such as nutrition, stress, toxicity, exercise, and drugs [31]. In the past decades, several investigations showed a crucial role of epigenetics in different physiological and pathological processes. Unlike genetic mutations, epigenetic changes are pharmacologically reversible. This aspect has encouraged many researchers to focus on epigenetic drugs (epidrugs) as cornerstone in management of many CV dysfunction [8–12].

3.1. DNA methylation

DNA methylation is a common mechanism used by cells to inhibit the accessibility of transcription machinery to gene promoters [33]. This modification consists on the binding of a methyl group to the 5′ position of cytosine residues in a dinucleotide Cytosine-phosphate-Guanine (CpG) by the DNA methyltransferases enzymes (DNMTs, including DNMT1, DNMT3A, and DNMT3B). In addition, demethylases belonging to 10–11 translocation (TET) family of DNA dioxygenases (TET1/2/3) also control the methylation status of the genome removing the methyl group from the methylated cytosines [34]. *S*-adenosylmethionine (SAM) is the donor of the methyl group in the DNMT-catalyzed reactions, while Fe (II)-, α-ketoglutarate- (α-KG), ascorbate-, and O_2 are the cofactors of the TET-catalyzed oxidation of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC). In contrast with methylation, demethylation of DNA usually activates transcription [34].

The expression levels of some genes encoding haemostatic proteins, including FVII [35], FVIII [36] and tissue-type plasminogen activator [37] have been shown to be regulated by DNA methylation mechanisms, thus altering their plasma concentrations in different conditions. NGS revolution opened the door to whole-genome DNA methylation analysis (MWAS) at a single-base-pair and single-cell resolution [38]. Nowadays, MWAS is a gold standard tool to discover novel genes and methylation marks potentially associated with environmental and genetic risk factors [38]. The research of methylation marks in peripheral blood DNA is considered an innovative approach to investigate complex disease etiology [39]. A pioneer study performed a MWAS by whole blood cells to identify a potential correlation between methylation marks and quantitative traits of coagulation cascade [40]. Another study reported that DNA methylation did not affect the FV Leiden polymorphism and did not contribute to explain the incomplete penetrance [41].

3.2. Histone modifications

Histone modifications include post-translational changes such as acetylation, methylation, phosphorylation, ubiquitination, and sumoylation affecting specific aminoacid residues of histone tails. The most studied are histone acetylation and methylation catalyzed by histone acetyl transferases (HATs) and histone deacetylases (HDACs), respectively. Acetylation of histone lysine residues increases chromatin accessibility resulting in activation of gene expression, whereas deacethylation induces a reduction of gene expression [42]. The synergistic regulation of HATs and HDACs establishes the level of histone acetylation contributing to the regulation of gene expression. Otherwise, the final effect of histone methylation varies according to the specific methylated residue and the number of added methyl groups. A particular histone modification, called citrullination, plays a key role in modulating the risk of VTE. After different stimuli, including infectious

agents, sterile inflammation, autoimmunity, and cancer, neutrophils undergo to apoptosis and expulsion of neutrophil extra-cellular traps (NETs) [43–45]. These complex are characterized by fibers of DNA combined with histones and proteins of granules, such as serine proteases, cathepsin G, and myeloperoxidase (MPO) [46]. After NETosis, the peptidyl arginine deiminase 4 (PAD4) over-expression converts specific arginine to citrulline residues of H3 and H4 histone tails [46]. Despite overexpression of PAD4 is not sufficient to induce NETosis, PAD4 activity is crucial for extensive chromatin decondensation. A large amount of NETs was identified within human atherosclerotic plaques and arterial/venous thrombi [45]. NETs showed high thrombotic potential because circulating histones enhanced generation of thrombin, secretion of Weibel-Palade body (WPB), adhesion of platelet and leucocyte as well as expression of platelet P-selectin, phosphatidylserine, and factor V/Va [44,[47]]. In addition, a cleavage mediated by neutrophil elastase inactivated the tissue factor pathway inhibitor (TFPI), a local endogenous anticoagulant [48]. Since fragment of NETs were present in circulation, they could be considered useful biomarkers of active VTE [44]. A case-control study reported that both circulating nucleosomes and neutrophil elastase-α1-antitrypsin complexes were associated with a three-fold risk of VTE [3,44].

Interestingly, a study demonstrated that circulating extracellular DNA (ceDNA) levels were elevated in VTE patients with a positive correlation with MPO levels [3]. Since MPO is stored in neutrophil granules, authors suggested that NETosis may be the source of the circulating DNA (Table 1). Otherwise, a recent study on 345 patients demonstrated that the levels of ceDNA and nucleosomes (but not NETs) were associated with VTE extents [46]. Furthermore, it has been observed that ceDNA level may be a useful prognostic biomarker associated with a poor patient outcome [49]. Thus, NET biomarkers might offer more accurate and non-invasive strategy for both diagnosis and risk stratification.

3.3. MicroRNAs

MiRNAs are endogenous single-stranded non-coding RNAs of 21–22 nucleotides. MiRNA biogenesis begins in nucleus and continues in cytosol performing a mechanism to regulate gene expression at post-transcriptional and translational level named RNA interference. These ribomolecules promote degradation or translational repression by imperfect base-pairing with the 3′UTR of target mRNAs. Besides intracellular molecules, a handful number of circulating miRNAs were detected in various types of bio-fluids correlating to different pathophysiological conditions [50]. Circulating miRNAs are remarkably stable by enwrapping into membrane vesicles (*i.e.* exosomes, microvesicles) [51]. Since circulating miRNA detection is repeatedly and non-invasive, they could represent useful diagnostic and predictive biomarkers [51] (Table 1). A recent study has reported that both miR-320a and miR-320b were significantly upregulated in VTE patients compared to controls, modulating cell migration, EC proliferation, apoptosis, and inflammation [52]. In addition, miRNA-320b was strongly correlated to D-dimer values in VTE patients than controls; thus, a simultaneous detection might improve accuracy in VTE diagnosis [52]. Another recent paper has shown that miR-495 down-regulation, by enhancing STAT3 expression, increase VTE risk [53]. Statistical analyses have confirmed miR-495 over-expression, t-PA, PAF, and PC as protective factors [53]. Moreover, miR-26a was significantly down-regulated inducing NF-kB signal in peripheral blood of VTE patients than controls [54]. Wang et al. have reported that circulating levels of miRNA-424–5p were significantly higher in VTE patients, while miR-136–5p expression resulted significantly lower as compared to patients without VTE [55]. Furthermore, Qin et al. have investigated the expression profile of 736 miRNAs in VTE patients and healthy subjects. Data reported that serum levels of miR-582, miR-195 and miR-532 were higher in VTE patients compared to controls, suggesting a possible role as diagnostic biomarkers in VTE disease [56] (Fig. 1).

4. Personalized therapy for VTE

4.1. Pharmacogenomics

In the era of personalized therapy, the goal is to fit therapeutic strategy to a personal genetic background. To make precision medicine applicable to all populations with different genetic/epigenetic background, it is imperative to identify causative factors that might become predictive biomarkers. In particular, warfarin response is largely influenced by individual genetic background as well as age, gender, drug-drug interactions, drug-food interactions, comorbidity, liver and renal function, and pregnancy [57]. Significant findings showed that SNPs in *CYP2C9*, encoding for warfarin metabolism enzyme, and in *VKORC1*, encoding for target of warfarin, explained a great part of inter-individual variability [57]. In particular, CYP2C9*2 (exon 3) and CYP2C9*3 (exon 7) decreased the activity of metabolic enzyme by approximately 30 and 80%, respectively [58]. Successively, a randomized clinical trial reported that warfarin dose requirement was reduced by 14 to 20% for CYP2C9*2 and 21 to 49% for CYP2C9*3 carriers [59]. Moreover, −1639G > A SNP in *VKORC1* promoter was associated with a two-fold lower levels of transcription in human liver. As consequence, a reduced warfarin maintenance dose was indicated in −1639G > A carriers [60]. In addition, VKORC1 mRNA contained a binding site for miR-133 and both molecules were co-expressed in human hepatocytes [61]. By sequencing, a SNP in *MIR133A2* correlating with warfarin dose requirement was observed. In detail, carriers of GA or AA genotype required a higher dose whereas carriers of GC genotype required a lower dose of warfarin compared to wild type genotype subjects [61]. These results highlighted a key role of

Table 1
Principal diagnostic and predictive epigenetic biomarkers associated to VTE.

	Source	Participants	Regulation	Clinical role	Ref.
Biomarker					
Circulating DNA	Plasma	94	Plasma DNA is elevated in patients with DVT and correlates with biomarkers of VTE.	Diagnostic/Predictive	[3]
NET fragments	Plasma	345	Increased levels of circulating nucleosomes and neutrophil activation, associated with a 3-fold risk of VTE.	Diagnostic/Predictive	[46]
MiRNAs					
miR-320a/b	Plasma	90	Circulating miR-320a/b is differentially expressed; miR-320b concentrations correlate to D-dimer levels in DVT.	Diagnostic	[49]
miR-495	Plasma	155	Down-regulation of miR-495 leads to increased VTE risk.	Diagnostic/Predictive	[50]
miR-26a	Whole Blood	85	miR-26a was significantly down-regulated in VTE patients *vs* controls.	Diagnostic	[51]
miR-424-5p, miR-136-5p	Plasma	238	Circulating miR-424–5p was significantly higher in VTE patients, while miR-136–5p was significantly reduced.	Diagnostic	[52]
miR-582, miR-195, miR-532	Serum	38	Serum levels of miR-582, miR-195 and miR-532 were higher in VTE patients compared to controls.	Diagnostic	[53]

Abbreviations: DVT: deep vein thrombosis; NET: neutrophil extra-cellular traps; VTE: venous thromboembolism.

Table 2
Clinical trials on genetics and pharmacogenomics of VTE.

NCT	Condition	Type of study	Participants	Purpose	Phase	Status	Ref.
NCT02828904	VTE	Observational/Prospective	27,620	To compare the VTE risk of COCs containing CMA 2 mg/EE 30 μg, compared to LNG 0.15 mg and EE 30 μg.	N/A	Recruiting	–
NCT03068923	VTE	Observational/Prospective	200	To compare biomarkers in children that develop poor VTE outcomes (recurrence, post-thrombotic syndrome and post-PE impairment) after an initial VTE with those that do not develop such outcomes.	N/A	Recruiting	–
NCT02904967	VTE	Interventional/Non-Randomized	1542	To identify new SNPs as genetic biomarkers of VTE recurrence by analyzing whole genome data of subjects with at least one thrombotic event.	N/A	Recruiting	–
NCT03114618	VTE; Cancer	Observational/Prospective	406	To evaluate a new clinic-genetic risk score for patients at high risk of VTE during chemotherapy.	N/A	Completed	[62,63]
NCT00856076	VTE; IUFD	Observational/Retrospective	1200	To investigate clinical, biochemical and genetic risk factors for VTE in pregnancy and related vascular complications.	N/A	Recruiting	[64]
NCT01006733	VTE	Interventional/Randomized	1598	To improve safety and effectiveness of clot prevention by customizing warfarin to each person genetic and clinical profile.	Phase 3	Completed	[61]
NCT01119300	VTE; AF	Interventional/Randomized	455	To determine whether a dosing algorithm containing genetic information increases the time within therapeutic INR range during anticoagulation therapy with each of warfarin, acenocoumarol and phenprocoumon compared to a dosing regimen that does not contain this information.	Phase 4	Completed	[60]
NCT00401414	PE; DVT; AF	Interventional/Non-Randomized	344	To find a better way based on genetic blood test to set the dose of warfarin.	N/A	Completed	[54,65]
NCT00511173	PE; DVT; AF	Interventional/Randomized	102	To compare clinician dosing and a pharmacogenetic algorithm in diagnosed patients requiring warfarin therapy.	Phase 4	Completed	–
NCT00927862	VTE	Interventional/Randomized	2415	To determine whether DNA analysis improves the efficiency of dosing and safety in patients in therapy with warfarin.	Phase 2 Phase 3	Completed	[55]
NCT01615705	DVT	Observational/Prospective	803	To evaluate whether biomarkers of inflammation, genetic thrombophilia and coagulation activation influence PTS onset in TVE patients.	N/A	Completed	[66]

The list is extracted from ClinicalTrials.gov. The studies with unknown status are not reported in the table.
Abbreviations: AF: atrial fibrillation; CMA: chlormadinone acetate; COC: combined oral contraceptive; DVT: deep vein thrombosis; EE: ethinylestradiol; IUFD: intrauterine fetal death; LNG: Levonorgestrel; N/A: not applicable; NCT: number of clinical trial; PE: pulmonary embolism; PTS: post-thrombotic syndrome; SNPs: single nucleotide polymorphism; VTE: venous thromboembolism.

epigenetics in warfarin response variability beyond of VTE genetic hereditability. The Food and Drug Administration included *CYP2C9* and *VKORC1* genotypes for optimizing warfarin dosing schedules [62]. Indeed, use of these genetic biomarkers could decrease time needed to achieve anticoagulation target levels as well as incidence of adverse effects [63]. A recent randomized clinical Genetic Informatics Trial (GIFT, NCT010006733) investigated whether genotype-guided dosing could improve safety of warfarin initiation (Table 2) [64]. Patients were genotyped for VKORC1-1639G > A, CYP2C9*2, CYP2C9*3, and CYP4F2 V433M SNPs and, successively, were randomized to genotype-guided or clinically guided warfarin strategy. Data showed that genotype-guided warfarin dosing reduced the risk of bleeding and VTE manifestation than clinically guided strategy [64].

In Table 2, we report studies focusing on prevention, diagnosis, and treatment of VTE generated from the website https://clinicaltrials.gov/. Clinical trials are grouped in two groups: genetics, pharmacogenetics aimed to establish novel biomarkers related to risk, diagnosis as well as response to warfarin therapy. Five clinical trials evaluated the role of novel SNPs modulating the risk of VTE. In particular, one current clinical trial (NCT02904967) evaluated the role of about 500,000 polymorphisms in VTE recurrence by analyzing whole genome data from MARTHA cohort composed of 1542 subjects with at least one episode of VTE documented. Another clinical trial (NCT03309293) investigated the role of DNA polymorphisms in 766 women using combined oral contraceptive (COC) reporting high frequency of thrombotic episodes. This study aimed to perform a predictive clinic-biological score of risk that could help to assess the individual risk of VTE before COC prescription. The study NCT03114618 analyzed whether the thrombosis clinic-genetic risk score could improve the detection of cancer patients at risk of VTE compared to the routinely used Khorana predictive model [65,66]. The trial NCT00018772 was designed to assess both clinical conditions as well as common genetic factors leading to more risk of VTE. Another study (NCT00856076) investigated the role of different SNPs in genes coding for specific coagulation, fibrinolysis, and inflammatory proteins as risk factors for VTE in pregnancy and intrauterine fetal death (IUFD) [67]. Regarding pharmacogenetics, four clinical trials investigating the role of precision medicine in warfarin dosing are reported. GIFT trial (NCT01006733) has been yet discussed [64]. One completed trial (NCT00401414) investigated the use of a genetic blood test based on SNPs in *CYP2C9* and *VKORC1* to identify the correct dose of warfarin and successful results were published [57,68]. Another study (NCT00511173) compared the standard clinical dosing to the use of pharmacogenetic algorithm in warfarin dose adjustment but results were not published. The study NCT00927862 determined whether DNA analysis could improve the efficiency of dosing and safety in patients who started warfarin therapy. Concerning inflammation, only a completed trial (NCT00401414) investigated if biomarkers of inflammation as CRP, ICAM, IL-6, and IL-10, genetic thrombophilia and coagulation activation (D-Dimer, F VIII, lupus anticoagulant, IgG, IgM) could influence post-thrombotic syndrome onset in VTE patients. Results showed that levels of CRP and IL-6 at diagnosis were related to clot extent, severity of VTE symptoms and signs [69].

4.2. Epidrugs

Recently, many investigators are focusing on epidrugs, such as metformin, fenofibrate, and statins as putative strategies to VTE management [70–72]. Here, we briefly describe the epidrugs-based molecular mechanisms. *In silico* analyses proposed that metformin operates as agonist of silent information regulator 1 (SIRT1), a nicotinamide adenine dinucleotide (NAD+)-dependent deacetylase belonging to class III deacethylases (HDAC3) [73]. Fenofibrate is a useful anti-hypertriglyceride agent acting as agonist of peroxisome proliferator-activated receptor alpha (PPARα) protein that induces SIRT1 expression, with consequent apoptosis of vascular adventitial fibroblasts

Table 3
Clinical trial on epidrugs in VTE.

NCT	Epidrug	Conditions	Type of study	Participants	Purpose	Phase	Status
Monotherapy							
NCT02679664	Rosuvastatin	VTE	Interventional/ Randomized	312	To determine if rosuvastatin can reduce PTS in VTE patients.	Phase 2	Recruiting
NCT01524653	Rosuvastatin	VTE; DVT; Neoplasms	Interventional/ Randomized	38	To assess the impact of rosuvastatin therapy on serum biomarkers (*i.e.* D-dimer) that indicate a risk for VTE, as well as safety and tolerance of rosuvastatin therapy in this population.	Phase 2	Completed
NCT01494090	Rosuvastatin	Hip fracture	Interventional/ Randomized	36	To evaluate the efficacy and safety of rosuvastatin in the prevention of arterial and venous vascular events and mortality after hip fracture.	Phase 3	Terminated
NCT01164540	Rosuvastatin	VTE; PE	Interventional/ Randomized	3000	To determine if treatment with rosuvastatin will decrease the risk of recurrent VTE and arterial thromboembolic events in patients with previous DVT or PE.	Phase 3	Withdrawn
Combined therapy							
NCT02331095	Atorvastatin/ Warfarin	VTE	Interventional/ Randomized	80	To determine the reduction of thrombin peak concentration and/or endogenous thrombin potential as well as VTE recurrence and mortality.	Phase 1/2	Recruiting
NCT02901067	Rosuvastatin/Aspirin	VTE; Wounds	Interventional/ Randomized	440	To assess the reduction of acute lung injury and VTE in patients with fibrinolysis shutdown.	Phase 2	Recruiting
NCT02285738	Simvastatin/Aspirin	Cancer	Interventional/ Randomized	42	To evaluate anti-platelet and statin therapy for the prevention of cancer-associated thrombosis.	Phase 1	Recruiting

Abbreviations: DVT: deep vein thrombosis; PE: pulmonary embolism; PTS: post-thrombotic syndrome; VTE: venous thromboembolism.

through deacetylation of forkhead box O1 (FoxO1) protein [74]. Statins are grouped in differentially structured type 1 (*e.g.*, simvastatin) and type 2 (*e.g.*, rosuvastatin, atorvastatin, fluvastatin) molecules, which act by inhibiting the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity, the limiting step of cholesterol synthesis in the liver [75]. Furthermore, statins, such as trichostatin A and valproic acid, present pleiotropic effects that may depend, in part, on their epigenetic effects, due to their ability in inhibiting HDAC activity. Currently, statins represent the gold standard cholesterol-lowering agent for prevention of different CV diseases [75]. In Table 3, we report some clinical trials testing the effects both of statins monotherapy and combined therapy in management of VTE.

4.3. Network medicine

The most challenging into application of personalized therapy arises from the innovative bioinformatic tools, which analyze disease phenotypes within the framework of molecular interaction (interactome) [76–78]. The main goal is to discover novel alterations from genotype to phenotype, thus ideally defining the causative basis linking candidate genes and diseases [76–78]. In the era of network medicine, the analysis of topological properties of protein-protein interactions (PPIs), regulatory, as well as co-expression networks guide research from cellular-molecular level of protein-protein interactions to correlation studies of gene expression in biological samples [76–78]. All of these networks can be viewed as maps where disorders are represented with localized perturbation within a specific module (pathway) of interactome.

A recent study explored the biomarkers associated with VTE by bioinformatics analyses [79]. Indeed, authors analyzed microarray data from Gene Expression Omnibus (GEO) database in patients with VTE (single or recurrent events) respect with controls. A total of 433 up-regulated and 222 down-regulated differentially expressed genes (DEGs) were obtained between single VTE and control samples and 625 up-regulated and 302 down-regulated DEGs were identified by comparing recurrent VTE data *vs* controls [79]. The overlap DEGs in the whole study population were mainly enriched in the pathways related to ribosome, cancer, and immune disease, while DEGs specific for recurrent VTE were enriched in several pathways, such as nod-like receptor signaling pathway. Protein-protein interaction (PPI) network analysis identified two clusters of VTE related-genes belonging to ribosomal protein family (*RPL9*, *RPL5*, *RPS20*, *RPL23*) and a set of proteins involved in the nod-like receptor signaling pathway. These findings provide helpful insights to find out novel biomarkers for the diagnosis and potential therapeutic modulator in VTE [79].

5. Conclusions

Nowadays, the knowledge of molecular pathways underling onset and progression of polygenic diseases is one of the major challenge in cardiovascular medicine. Although epigenetic mechanisms, such as citrullination and some miRNAs are associated with VTE, no validated epigenetic biomarkers are routinely used in the clinical practice. Despite the potential diagnostic value, miRNA studies in VTE have not been consistent; indeed many different entities have been proposed, in part due to several difference in study design and data collection. Consequently, the potential diagnostic or therapeutic value of these molecules should be tested in studies with a larger number of patients. In the era of personalized therapy, several clinical trials are investigating the putative role of statins as additional therapeutic agents in the management of VTE patients. Furthermore, pharmacogenomic studies evaluated the magnitude of individual genetic signatures to help physicians in determining warfarin dose requirement, in order to improve safety and effectiveness of VTE treatment. To date, the reductionist approach has been largely successful and responsible for most of drugs; however, given the polygenic nature of VTE, the network-based

approach may aid to prioritize novel VTE candidate genes and design innovative drug targets able to improve the personalized therapy [80].

Declarations of interest

None.

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Author contributions

All authors conceived and drafted the study, and analyzed and interpreted the data. All authors had full access to all of the data in the study and can take responsibility for the integrity of the data and the accuracy of the data analysis.

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