useful to inhibit thrombosis on artificial medical surfaces. Is too much inhibition of contact activation and BK formation potentially deleterious? *Kgn1*$^{-/-}$ mice exist without BK and obvious consequence. The clinical use of α_1AT-SMTR/V and α_1AT-SLLR/V inhibitors may induce the mild medical hemophilia C (FXIa inhibition), a mild bleeding disorder. This activity may be useful for contact activation-induced thrombosis prevention.

The 2 articles highlighted here show the novelty and diversity of therapeutic development in the areas of hemostasis and contact activation. It is exciting to observe how simple amino acid substitutions make a serpin elastase inhibitor (α_1AT) into a potent antithrombin, activated protein C inhibitor, or anti-PKa/FXIIa inhibitor.[2,4,11] It is important to learn that downregulation of PN-1 alone improves thrombin generation.

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THROMBOSIS AND HEMOSTASIS

Comment on Lindström et al, page 1645

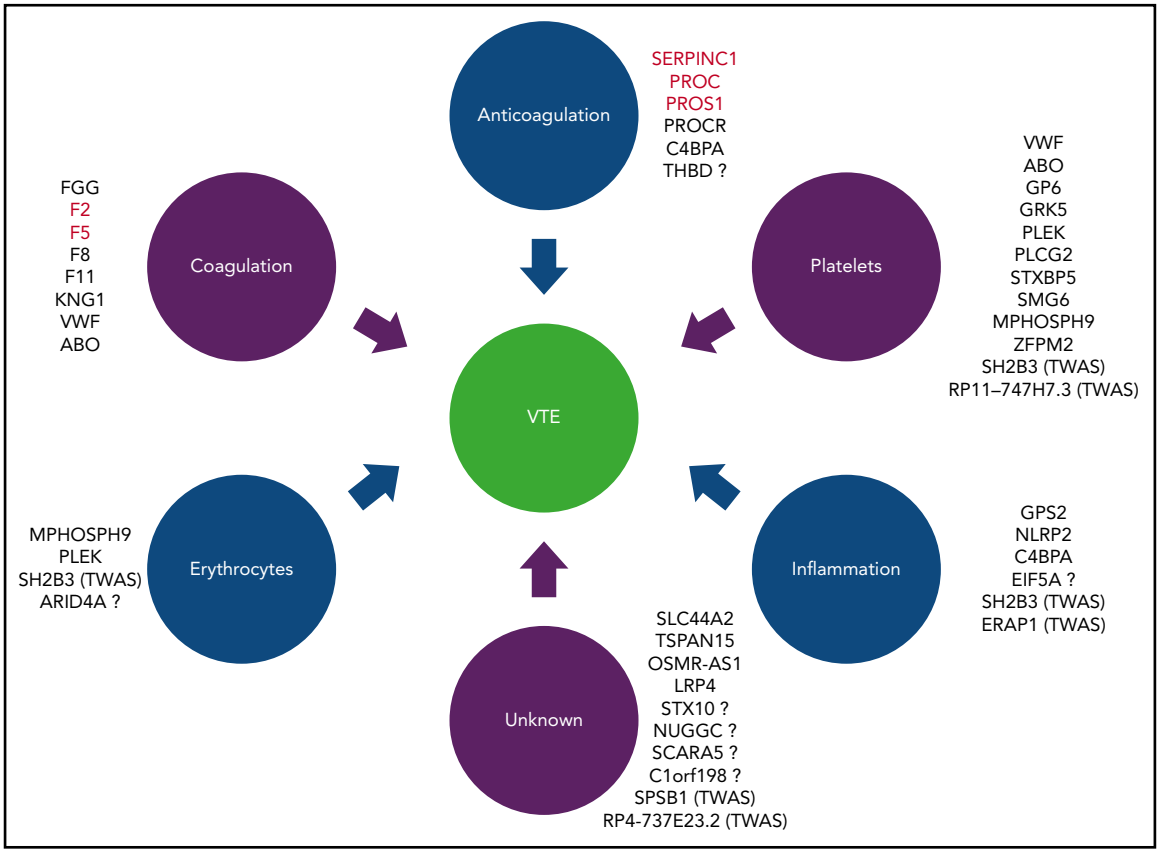
Genetics of venous thromboembolism revised

Bengt Zöller | Lund University

In this issue of *Blood*, Lindström and colleagues[1] report genomic and transcriptomic association of 16 novel susceptibility loci for venous thromboembolism (VTE). Moreover, Mendelian randomization causally linked blood traits to thrombosis.

Familial aggregation of VTE was recognized in 1905 by Briggs,[2] but it was not until 1965 that Egeberg identified the first genetic risk factor for VTE (ie, inherited deficiency of antithrombin [SERPINC1]) (see figure).[3] In 1981 and 1984, inherited deficiencies of protein C (PROC) and protein S (PROS1) were recognized as risk factors for VTE. However, it was not until the discovery of resistance to activated protein C (APC resistance) by Dahlbäck et al in 1993 that it became evident that genetic factors are common risk factors of VTE.[4] APC resistance was linked to a mutation in the factor V gene (F5), factor V Leiden (rs6025).[5] In 1996, Poort et al reported a common genetic variation (rs1799963) in the 3′-untranslated region of the prothrombin gene (F2) is associated with elevated plasma prothrombin levels and VTE risk.[6] These 5 inherited defects are called major thrombophilias, and all involve coagulation or anticoagulant genes. No new major thrombophilia has been discovered since 1996. Instead, genome-wide association studies (GWASs) have discovered a number of VTE-associated loci, reviewed by Trégouët and Morange.[7] The GWAS-discovered risk variants are weak risk factors for VTE but are prevalent in the population. Before the present study, all genes linked to VTE were directly or indirectly linked to the coagulation system (FGG, F2, F5, F8, F11, KNG1, VWF, ABO), anticoagulation pathways (SERPINC1, PROC, PROS1, PROCR, THBD), or platelets (VWF, GP6, ABO, STXBP5, ZFPM2), although some loci (SLC44A2, TSPAN15, LRP4) had no known biological link to VTE.

The present study by Lindström et al is the largest meta-analysis of GWAS data for VTE, including 18 studies with 30 234 VTE cases and 172 122 controls. The study is the first *trans*-ancestry GWAS of VTE. It is also the first transcriptome-wide association study (TWAS). The GWAS identified 11 newly associated genetic loci (C1orf198, PLEK, OSMR-AS1, NUGGC/SCARA5, GRK5, MPHOSPH9, ARID4A, PLCG2, SMG6, EIF5A, and STX10). The TWAS identified 5 additional genetic loci using imputed gene expression in whole blood (SH2B3, SPSB1, RP11-747H7.3, RP4-737E23.2) and in liver (ERAP1). Some previous associations could not be confirmed in the present GWAS, such as the THBD loci.[7] The present study gives important contributions to the functions of genes involved in the pathogenesis of VTE (see figure). In the figure, the genes are grouped after potential links to VTE. The strongest genetic risk factors for VTE (ie, classical thrombophilia) are all related to coagulation (F2 and F5) or the anticoagulation



Genes associated with VTE. Genes are grouped according to biological links to VTE: coagulation, anticoagulation, platelets, erythrocytes, inflammation, and unknown. Genes associated with major thrombophilias are marked in red. Question marks denote not replicated genes. TWAS shows that the gene was identified with transcriptome-wide association study.

system (SERPINC1, PROC, PROS1). However, the present study adds several loci all involved directly or indirectly in platelet biology but also loci involved in erythrocyte biology or inflammation. The present study also used Mendelian randomization and showed that blood traits, especially related to platelets, are causally associated with VTE risk. Thus, platelets that before have been implicated mostly in arterial thrombosis are also of importance for VTE. Thus, the present study suggests that the formed elements in the blood need more attention from researchers in the field of VTE.

Inflammatory conditions, such as infection and immune-mediated diseases, have been linked to VTE,[8] but the present study is the first to link several genes related to inflammation to VTE underlining the importance of inflammation in VTE pathobiology. Whether inflammation per se is a driver of thrombosis or whether inflammation is due to its effects on coagulation, anticoagulation, and platelets remains to be determined.

The present study also tested a new genetic risk score (GRS) based on 37 loci. The GRS constructed from these variants will become a useful research tool due to its strong impact on VTE risk, although its clinical usefulness remains to be determined. For instance, a recent study has shown that family history of VTE affects the risk of both VTE and major bleeding following hip and knee replacement surgical procedures.[9] It could therefore be worthwhile to determine the value of the new GRS for prediction of both VTE and bleeding.

The heritability on the observed scale due to genotyped variants was 23%. Transforming this to the liability scale assuming a disease prevalence of 0.5% resulted in a heritability of 15%. This is much lower than the estimated heritability of 40% to 60% from studies of families, twins, siblings, and half-siblings.[2] The cause of the remaining heritability is unclear and might be due to rare variants, although a recent exome study by Lindström et al of 8332 cases and 16 087 controls of European ancestry and 382 cases and 1476 controls of African American ancestry found no significant novel rare variants.[10] However, the design of exome studies needs to be developed because burden analysis could not confirm that the 3 major thrombophilia genes (SERPINC1, PROS1, and PROC) are associated with VTE.[10] Only the Ser501Pro variant of PROS1 (PS Herleen mutation; rs121918472) with a minor allele frequency of 0.005 could be confirmed. Family studies using new strategies might be an option. In the present study, variants in novel loci were all intronic or intergenic. Maybe-whole genome sequencing will be an important way for mapping the missing heritability of VTE. As in the present study, using expression data from Genotype-Tissue Expression might be another option for the detection of functional variants.

With the present milestone study by Lindström et al, the list of VTE-associated loci has grown considerably. Genes have been identified that are linked not only to coagulation and anticoagulation but also

to platelets, erythrocytes, inflammation, or genes without any so far known biological link with thrombosis (see figure). The results will be important not only for future genetic and clinical studies but also for directing future function-structure studies of the molecular cause of VTE.

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TRANSPLANTATION

Comment on Koehn et al, page 1670

We didn't start the fire, MDSC inflammasome signaling in GVHD

Nataliya Prokopenko Buxbaum | National Institutes of Health

In this issue of *Blood*, Koehn et al investigate how activation of the nucleotide-binding domain and leucine-rich repeat (NLR) pyrin family domain 3 (NLRP3) inflammasome pathway influences the function of myeloid-derived suppressor cells (MDSCs) in the setting of acute graft-versus-host disease (aGVHD).[1]

The authors use a wide range of genetic and pharmacologic tools to dissect the contributions of the inflammasome signaling pathway components to loss of suppressive function in MDSCs. Although T cells are the main drivers of GVHD, MDSC suppression of alloreactivity is a promising strategy for inhibiting GVHD because MDSCs have the potential to suppress $CD4^+$ and $CD8^+$ T cells, as well as natural killer and natural killer T cells.[2] Efficacy of MDSCs generated ex vivo in aGVHD was previously explored, but efficacy was limited because MDSCs lost their suppressive ability when they were placed into a highly inflammatory milieu within the hematopoietic stem cell transplant (HSCT) recipient. MDSC function or its loss is highly dependent on environmental cues. To delineate the environmental and intrinsic mechanisms of MDSC activation, Koehn et al explored the role of the NLRP3 inflammasome pathway in an aGVHD major histocompatibility mismatch mouse model.

MDSCs are a diverse population of immature myeloid cells produced during chronic inflammatory states that have the ability to suppress anti-tumor immunity as well as inflammation in a variety of clinical settings.[2,3] Dampening MDSC suppressor function is an attractive strategy for optimizing cancer immunotherapy because MDSCs are implicated in tumor progression and metastasis. Conversely, enhancing or sustaining MDSC suppressive function would be beneficial in inhibiting inflammation associated with GVHD. MDSCs can be targeted through a variety of pharmacologic approaches. However, to successfully maintain MDSCs in the desired suppressive state in vivo, it is essential to understand the pathways that direct MDSC behavior in the context of the specific biological setting.

Previous investigations in aGVHD identified the NLRP3 inflammasome as a key pathway for MDSC alloimmune activation (see figure).[4] MDSCs can be generated from bone marrow stem cells in an interleukin-13 (IL-13) culture system, and they are able to ameliorate aGVHD after being infused into the host.[5,6] The effect on aGVHD is modest, however, and repeated infusions can further improve outcomes but do not fully abrogate aGVHD. MDSC loss of suppressive function after HSCT occurs when the conditioning regimen induces the release of adenosine triphosphate (ATP) into the extracellular compartment (see figure). The release of ATP results from cell damage induced by pretransplant conditioning, particularly irradiation. ATP binding to the P2x7 receptor (P2x7R) on MDSCs results in downstream NLPR3 inflammasome activation. The canonical inflammasome activation then initiates a cascade of proinflammatory effects that fuel aGVHD. Koehn et al show that P2x7 knockout (KO) or inhibition of ATP binding to the receptor reduced inflammasome activation. This was demonstrated with extracellular ATP depletion via apyrase and pharmacologically via administration of A-438079, a highly selective P2x7R inhibitor. Both approaches resulted in improved post-HSCT survival in the aGVHD model, with important clinical implications.

The downstream result of NLRP3 inflammasome activation is caspase-1–mediated